# MOLECULAR CHARACTERIZATION OF THE *ARABIDOPSIS THALIANA* HISTIDINE KINASE 1 AND TRANSITIONS FROM THE MULTISTEP PHOSPHORELAY SYSTEM TO SER/THR/TYR PHOSPHORYLATION

**DISSERTATION**

der Mathematisch-Naturwissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr. rer. nat.)

vorgelegt von
Rebecca Dautel
aus Filderstadt

Tübingen
2016

Gedruckt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Eberhard Karls Universität Tübingen

| | |
|---|---|
| Tag der mündlichen Qualifikation: | 24. März 2017 |
| Dekan: | Prof. Dr. Wolfgang Rosenstiel |
| 1. Berichterstatter: | Prof. Dr. Klaus Harter |
| 2. Berichterstatter: | Prof. Dr. Claudia Oecking |
| 3. Berichterstatter: | Prof. Dr. Jan Hejátko |

# TABLE OF CONTENT

# FIGURE LIST

# TABLE LIST

# ABBREVIATION LIST

| | |
|---|---|
| aa | amino acid |
| ABA | abscissic acid |
| ABI | ABSCISSIC ACID INSENSITIVE |
| ACC | 1-aminocyclopropane-1-carboxylic acid |
| Ade | adenine |
| AHA | $H^+$-ATPases |
| AHK | *Arabidopsis thaliana* sensor hybrid histidine kinase |
| ahk1.m | *ahk1-3* treated for 10min with 0.3M mannitol |
| ahk1.man | *ahk1-3* treated for 10min with 0.3M mannitol |
| AHP | *Arabidopsis thaliana* phosphotransferprotein |
| Ala | alanine |
| aLRL | average lateral root length |
| Amp | Ampicillin |
| APS | ammonium persulfate |
| ARF | AUXIN RESPONSE FACTOR |
| ARG1 | ALTERED RESONSE TO GRAVITY 1 |
| ARR | *Arabidopsis thaliana* response regulator |
| Asp | aspartate |
| ATP | adenosine triphosphate |
| AUX | auxin |
| BAK1 | BRI1-ASSOCIATED RECEPTOR KINASE 1 |
| BAS1 | PHYTOCHROME B ACTIVATION TAGGED SUPPRESSOR 1 |
| BCIP | 5-bromo-4-chloro-3-indolylphosphate-p-tuloidin |
| BES1 | BRI1-EMS-SUPPRESSOR 1 |
| bHLH | basic helix-loop-helix |
| BIN2 | BRASSINOSTEROID-INSENSITIVE 2 |
| BIR | BAK1-INTERACTING RECEPTOR-LIKE KINASE |
| BKI1 | BRI1 KINASE INHIBITOR 1 |
| BL | brassinolide |
| bp | basepairs |
| BR | brassinosteroid |
| BRI1 | BRASSINOSTEROID-INSENSITIVE 1 |
| BSK | BR-SIGNALING KINASE |
| BSL | BRI1 SUPRESSOR 1-LIKE |
| BSU1 | BRI1-SUPPRESSOR 1 |
| BTB | BROAD COMPLEX/TRAMRACK/BRICK-A-BRACK |
| bZIP | basic leucine zipper |
| BZR1 | BRASSINAZOLE-RESISTANT 1 |
| *CaMV* | *Cauliflower Mosaic Virus* |
| CBL | Calcineurin B-like |
| CDG1 | CONSTITUTIVE DIFFERENTIAL GROWTH 1 |
| CDPK | $Ca^{2+}$ DEPENDENT PROTEIN KINASE |
| CHASE | extracytosolic cyclases/histidine kinase associated sensor extracellular |
| CIPK | CBL-interacting protein kinase |
| CK | cytokinin |
| CK2 | CASEIN KINASE 2 |

| | |
|---|---|
| CKA | CASEIN KINASE ALPHA CHAIN |
| CKI1 | CYTOKININ INSENSITIVE 1 |
| Cm | Chloramphenicol |
| CNGC | CYCLIC NUCLEOTIDE GATED CHANNEL |
| Col-0 | Columbia-0 |
| COP1 | CONSTITUTIVE PHOTOMORPHOGENIC 1 |
| CPD | CONSTITUTIVE PHOTOMORPHOGENIC DWARF |
| CRL | CULLIN-RING ligase |
| CRLK1 | CALCIUM/CALMODULIN-REGULATED RECEPTOR-LIKE KINASE 1 |
| CRY | CRYPTOCHROME |
| C-terminus | carboxy terminus |
| CTP | cytidintriphosphat |
| CTR1 | CONSTITUTIVE TRIPLE RESPONSE |
| Cub | carboxy terminal part of ubiquitin |
| CUL | CULLIN |
| CYP735A1 | CYTOCHROME P450; FAMILY 735; POLYPEPTIDE 1; SUBFAMILY A |
| dag | days after germination |
| dat | days after transfer |
| DDB1 | DAMAGED DNA BINDING 1 |
| DET1 | DE-ETIOLATED 1 |
| DMSO | dimethylsulfoxid |
| DNA | deoxyribonucleic acid |
| dpi | dots per inch |
| DWA1 | DWD HYPERSENSITIVE TO ABA 1 |
| DWD | DDB1-BINDING/WD-40 DOMAIN CONTAINING |
| DWF | DWARF |
| ED | extracellular domain |
| EIN | ETHYLENE INSENSITIVE |
| ER | endoplasmic reticulum |
| ERS | ETHYLENE RESPONSE SENOR |
| ET | ethylene |
| *et al.* | et alii (and others) |
| EtOH | ethanol |
| ETR | ETHYLENE RESPONSE |
| exp. | experiment |
| FAD | flavin adenine dinucleotide |
| FDR | false discovery rate |
| FER | FERONIA |
| FHY3 | FAR-RED ELONGATED HYPOCOTYL 3 |
| fig. | figure |
| flg22 | flagellin22 |
| FLIM | Fluorescence lifetime imaging |
| FLS2 | FLAGELLIN-SENSITIVE 2 |
| FMN | flavin mononucleotide |
| FRET | Förster resonance transfer |
| FyPP | PHYTOCHROME-ASSOCIATED PROTEIN PHOSPHATASE |
| GA | gibberellic acid |
| Gent | Gentamycin |
| GFP | green fluorescent protein |
| GID1 | GA-INSENSITIVE DWARF 1 |
| GRAS | GA INSENSITIVE; REPRESSOR OF *ga1-3*; SCARECROW |
| GRF | GROWTH-REGULATING FACTOR |

| | |
|---|---|
| GTG | PROTEIN-COUPLED RECEPTOR-TYPE G PROTEIN |
| GTP | guanosintriphosphat |
| GUS | β-glucuronidase |
| His | histidine |
| HK | histidine kinase |
| HOG | HIGH OSMOLARITY GLYCEROL |
| HPLC | high-performance liquid chromatography |
| HY5 | ELONGATED HYPOCOTYL 5 |
| Hyg | Hygromycin |
| IAA | indole-3-acetic acid |
| ICP | intracellular part |
| $InsP_6$ | myo-inositol hexakisphosphate |
| IPTG | isopropyl-β-D- thiogalactopyranosid |
| JA | jasmonate |
| Kan | Kanamycin |
| kDa | kilo Dalton |
| KEA | $K^+$ EFFLUX ANTIPORTER |
| LB | Luria-Bertani |
| LC | liquid chromatography |
| LD | long day |
| LiAc | lithium actetate |
| LOG7 | LONELY GUY 7 |
| LOV | LIGHT, OXYGEN, VOLTAGE |
| LRD | lateral root density |
| LRP | lateral root primordium |
| LRR-RK | leucine-rich repeat receptor kinase |
| LUC | luciferase |
| MAP | MITOGEN-ACTIVATED PROTEIN |
| mbSUS | mating-based split-ubiquitin system |
| mC | mCherry |
| MeJA | methyl-jasmonate |
| MES | 2-(N-morpholino)ethanesulfonic acid |
| Met | methionine |
| met. | metabolic |
| MKK | MITOGEN-ACTIVATED PROTEIN KINASE KINASE |
| MKKK | MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE |
| MPK | MITOGEN-ACTIVATED PROTEIN KINASE |
| MRVA | main root vector angle |
| MS | Murashige and Skoog |
| MS | mass spectrometry |
| MSL | MscS-LIKE |
| MSP | multistep phosphorelay |
| NASC | Nottingham Arabidopsis Stock Center |
| NBT | nitro blue tetrazolium |
| NIT2 | NITRILASE 2 |
| NLS | nuclear localization signal |
| NoLRs | number of lateral roots |
| Nos-0 | Nossen-0 |
| NPA | 1-N-naphthylphthalamic acid |
| NPR1 | NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 |
| N-terminus | amino terminus |
| Nub | amino terminal part of ubiquitin |

| | |
|---|---|
| OD | optical density |
| PAMP | pathogen-associated molecular pattern |
| PAS | Per-Arnt-Sim |
| PCR | polymerase chain reaction |
| Pcz | propiconazole |
| PHOT | PHOTOTROPIN |
| PHY | PHYTOCHROME |
| PI | propidium iodide |
| PIF | PHYTOCHROME INTERACTING FACTORS |
| PIN | PIN-FORMED |
| PIP | PLASMA MEMBRANE INTRINSIC PROTEIN |
| PP2A | protein phosphatase 2A |
| PP2C | protein phosphatase 2C |
| PYL | PYRABACTIN RESISTANCE-LIKE |
| PYR | PYRABACTIN RESISTANCE |
| RAB18 | RESPONSIVE TO ABSCISSIC ACID 18 |
| RALF | rapid alkalinization factor |
| RBX1 | RING-BOX 1 |
| RCAR | REGULATORY COMPONENT OF ABA RECEPTORS |
| RD29B | RESPONSIVE TO DESICCATION 29B |
| REC | receiver |
| RFP | red fluorescent protein |
| Rif | rifampicin |
| RING | REALLY INTERESTING NEW GENE DOMAIN |
| RLK | RECEPTOR-LIKE KINASE |
| RLP | RECEPTOR-LIKE PROTEIN |
| RNA | ribonucleic acid |
| ROS | reactive oxygen species |
| rpm | rounds per minute |
| SA | salicylic acid |
| SAR | systemic acquired resistance |
| SAUR | SMALL AUXIN UP-RNA |
| SCF | SKP1, CUL1, F-box protein |
| SD | short day |
| SDS-PAGE | sodium dodecyl sulfate polyacrylamid gel electrophoresis |
| Ser | serine |
| SIRK1 | SUCROSE-INDUCED RECEPTOR KINASE 1 |
| SKP1 | S-PHASE KINASE-ASSOCIATED PROTEIN 1 |
| SL | strigolactone |
| SnRK | SUCROSE NONFERMENTING 1-RELATED PROTEIN KINASE |
| SOB | super optimal broth |
| SOB7 | SUPPRESSOR OF PHYB-4 7 |
| SOS | SALT OVERLY SENSITIVE |
| SPA | SUPPRESSOR OF PHYA-105 |
| Spec | Spectinomycin |
| SPS1F | SUCROSE PHOSPHATE SYNTHASE 1F |
| TCS | two-component signaling |
| T-DNA | transfer DNA |
| TEMED | tetramethylethylenediamine |
| TF | transcription factor |
| Thr | threonine |
| TIR1 | TRANSPORT INHIBITOR RESPONSE 1 |

| | |
|---|---|
| TM | transmembrane domain |
| TPL | TOPLESS |
| TPR | TOPLESS-RELATED |
| Trp | tryptophane |
| TTP | thymidintriphosphat |
| Tyr | tyrosine |
| Ura | uracile |
| UV-A | ultraviolet-A |
| UV-B | ultraviolet-B |
| (v/v) | volume per volume |
| (w/v) | weight per volume |
| WAK | WALL-ASSOCIATED KINASE |
| Ws-2 | Wassilewskija-2 |
| wt | wildtype |
| wt.m | Ws-2 treated for 10min with 0.3M mannitol |
| wt.man | Ws-2 treated for 10min with 0.3M mannitol |
| Y2H | yeast two-hybrid |
| YEB | yeast extract broth |
| YUC | YUCCA |
| ZTL | ZEITLUPE |
| β-ME | β-mercaptoethanol |
| $\psi_w$ | water potential |

# SUMMARY

The *Arabidopsis thaliana* sensor hybrid histidine kinase AHK1 was suggested to work as osmosensor in plants (Urao *et al.*, 1999; Reiser *et al.*, 2003; Tran *et al.*, 2007; Wohlbach *et al.*, 2008). Several studies could show, that AHK1 indeed is involved in the regulation of osmotic stress adjustment (Urao *et al.*, 1999; Reiser *et al.*, 2003; Tran *et al.*, 2007; Wohlbach *et al.*, 2008; Kumar *et al.*, 2013). Nevertheless there were opposing results whether AHK1 works as positive or negative regulator of osmoregulation (Tran *et al.*, 2007; Wohlbach *et al.*, 2008; Kumar *et al.*; 2013).

In this study it could be revealed, that under defined conditions AHK1 in fact acts as positive regulator of osmoregulation. It is shown that this is highly influenced by various factors, including variations in temperature. A consistent phenotype was found in etiolated seedlings for *ahk1* knock down mutants in the *Arabidopsis thaliana* ecotypes Ws-2 and Nos-0. Col-0 did not show this alteration under normal growth conditions which might be in part due to the contribution of phyD to hypocotyl elongation in etiolated seedlings (Aukerman *et al.*, 1997). Still, in etiolated seedlings a physiological function of the AHK1-BAK1 interaction could be revealed. Furthermore, a trend of an AHK1-dependent altered response of *Arabidopsis thaliana* to infection with the necrotophic fungus *Alternaria brassicicola* could be observed which confirms this interaction.

The analysis of the phosphoproteome of *ahk1-3* and the wildtype Ws-2 showed a tremendous transition from the AHK1-induced His-to-Asp multistep phosphorelay to Ser/Thr/Tyr phosphorylation cascades after mock as well as after mannitol treatment. Such a transition was also observed in the analysis of the phosphoproteome of *ahk2 ahk3* and the wildtype Col-0 after mock and after kinetin treatment. Thereby the overlap of the quantified phosphopeptides in the phosphoproteomes of *ahk1-3*/Ws-2 and *ahk2 ahk3*/Col-0 was surprisingly small.

With the analysis of the phosphoproteome of *ahk1-3* and Ws-2 proteins were identified which indeed showed interaction with AHK1 in subsequent protein-interaction assays. Furthermore, the analysis of the phosphoproteome together with the results of phenotyping experiments led to the establishment of a putative AHK1-dependent regulatory network which could now be further investigated.

Due to the generation of a homology model for the structure of the extracellular domain of AHK1, the suggested mechano-sensitive signal perception mechanism is doubted. For further verification it was achieved to express the extracellular domain of AHK1 to go for ligand fishing and crystallization.

# ZUSAMMENFASSUNG

Bisher wurde angenommen, dass die *Arabidopsis thaliana* Sensor Hybrid Histidine Kinase AHK1 als möglicher Osmosensor fungiert (Urao *et al.*, 1999; Reiser *et al.*, 2003; Tran *et al.*, 2007; Wohlbach *et al.*, 2008). Mehrere Studien haben gezeigt, dass AHK1 tatsächlich an der Regulation der Anpassung der Pflanze an osmotischen Stress beteiligt ist (Urao *et al.*, 1999; Reiser *et al.*, 2003; Tran *et al.*, 2007; Wohlbach *et al.*, 2008; Kumar *et al.*, 2013). Jedoch wurden unterschiedliche Ergebnisse erzielt in Hinblick darauf, ob AHK1 als positiver oder negativer Regulator in der Regulation bei osmotischem Stress beteiligt ist (Tran *et al.*, 2007; Wohlbach *et al.*, 2008; Kumar *et al.*; 2013).

In dieser Studie konnte gezeigt werden, dass AHK1 unter definierten Bedingungen als positiver Regulator in der Regulation von osmotischem Stress wirkt. Es wird gezeigt, dass dies stark von verschiedenen Faktoren wie beispielsweise Unterschiede in der Temperatur beeinflusst wird. Ein beständiger, reproduzierbarer Phänotyp wurde für etiolierte Keimlinge der *ahk1 knock down* Mutanten in *Arabidopsis thaliana* der Ökotypen Ws-2 und Nos-0 gefunden. Im Ökotyp Col-0 konnte dies nicht im gleichen Umfang gezeigt werden was teilweise auf dem Fehlen von funktionellem Phytochrom D in Ws-2 beruhen könnte, das erwiesenermaßen am Elongationswachstum des Hypokotyls beteiligt ist (Aukerman *et al.*, 1997). Außerdem konnte in etiolierten Keimlingen ein physiologischer Zusammenhang der Interaktion von AHK1 mit BAK1 gezeigt werden. Dieser physiologische Zusammenhang wurde durch die Tendenz einer AHK1-abhängigen veränderten Reaktion von *Arabidopsis thaliana* auf die Infektion mit *Alternaria brassicicola* bekräftigt.

Die Analyse des Phosphoproteoms von *ahk1-3* und dem Wildtyp Ws-2 zeigte einen massiven Übergang vom Histidin-zu-Aspartat Phosphatgruppenrelais zu klassischen Serin/Threonin/Tyrosin Phosphorylierungskaskaden nachdem die Keimlinge einer Scheinbehandlung und der Behandlung mit osmotischem Stress, der mit Hilfe von 0.3M Mannitol appliziert wurde, unterzogen wurden. Ein derartiger massiver Übergang konnte ebenfalls für *ahk2 ahk3* und Col-0 nach einer Scheinbehandlung und der Behandlung mit Kinetin gezeigt werden. Der Überlapp der quantifizierten phosphorylierten Peptide war dabei erstaunlich gering.

Durch die Analyse des Phosphoproteoms von *ahk1-3* und Ws-2 konnten Proteine identifiziert werden, die in nachfolgenden Interaktionsstudien Interaktion mit AHK1 zeigten. Darüber hinaus führte die Analyse des Phosphoproteoms zusammen mit den Ergebnissen der Phänotypisierung zu der Erstellung eines putativ AHK1-abhängigen regulatorischen Netzwerks, das nun weiterführend untersucht und charakterisiert werden kann.

Auf der Grundlage des Homologiemodells für die Struktur der extrazellulären Domäne der AHK1 kann vermutet werden, dass die Perzeption eines Signals von AHK1 nicht mechanisch abläuft sondern dass es sich bei dem Signal um einen niedermolekularen Liganden handeln könnte. Um dies nachzuweisen wurden erste Ergebnisse in der Expression der extrazellulären Domäne der AHK1 erzielt die in weiterführenden Experimenten der Kristallisierung und dem Fischen nach dem putativen Liganden dienen soll.

# 1 INTRODUCTION

Plants are sessile organisms which are continuously exposed to various environmental stimuli to which they have to adapt to (Suzuki *et al.*, 2016; Pekárová *et al.*, 2016). They evolved several pathways for signal perception and signal transduction which enable them to sense, process and respond to diverse external stimuli (Pekárová *et al.*, 2016). These pathways can act independently but cooperation and cross-talk between them is essential for the integration of various signals and to effect the proper adaptive responses (Pekárová *et al.*, 2016). Sensing of environmental stimuli in general leads to post-translational modifications of proteins to transduce the signal to the nucleus to cause a change in transcription factor (TF) activity to achieve adapted gene expression which in turn leads to an adjustment to the altered environmental surrounding (Deribe *et al.*, 2010; Appleby *et al.* 1996; Pekárová *et al.*; 2016). There are diverse post-translational modifications which are involved in signal transduction. For instance sumoylation and ubiquitination of proteins controls their localization and activity but primarily targets them for proteasomal degradation (Miura *et al.*, 2009; Hua and Vierstra, 2011). Furthermore, phosphorylation of proteins is involved in the regulation of protein activity, protein localization and protein-protein interactions and thereby plays a central role in signal transduction pathways (Vu *et al.*, 2016). In proteins serine (Ser), threonine (Thr), tyrosine (Tyr), histidine (His) and aspartate (Asp) residues can be phosphorylated (Sanders *et al.*, 1989; Appleby *et al.*, 1996; Pekárová *et al.*, 2016). Ser/Thr/Tyr phosphorylation is connected to classical phosphorylation cascades which lead to an amplification of the signal (Posas *et al.*, 1996; Droillard *et al.*, 2004). For instance, a typical phosphorylation cascade occurs with the activation of MITOGEN-ACTIVATED PROTEIN (MAP) KINASEs (MPKs) in which MAP kinase kinase kinases (MKKKs) phosphorylate and activate MAP kinase kinases (MKKs) which in turn phosphorylate MPKs (Droillard *et al.*, 2004). Instead, His and Asp phosphorylation is connected to the His-to-Asp phosphorelay in the multistep phosphorelay (MSP) system which does not lead to a signal amplification which is due to the transfer of one phosphate (Posas *et al.*, 1996). Ser, Thr and Tyr phosphorylation is more stable than His and Asp phosphorylation and a direct transfer of the phosphate group from His and Asp to Ser, Thr or Tyr or the way around is biochemically not possible (Sanders *et al.*, 1989).

In the beginning of the 20$^{th}$ century it has been proven that growth in plants is under control of special growth substances or hormones and that they work at “concentrations of almost unbelievable lowness” in the range of nanomolar concentrations (Paál, 1918; Thimann, 1938). Hormones have been defined as chemical messengers which are produced in one cell but modulate the cellular processes in another cell (Taiz and Zeiger, 2010). This is linked to signal perception by receptors and signal transduction which leads to the adaption of cellular processes and which also includes phosphorelay systems as well as phosphorylation cascades (Taiz and Zeiger, 2010; Wang *et al.*, 2012b; Pekárová *et al.*; 2016). So far, ten types of plantal hormones have been identified to regulate growth, development and stress responses: Auxins, cytokinins, gibberellic acids (GAs), ethylene, abscissic acid (ABA), brassinosteroids (BRs), jasmonates (JAs), salicylic acid (SA), strigolactones (SLs) and small polypeptides (Taiz and

Zeiger, 2010). Furthermore, second messengers like for instance $Ca^{2+}$, cyclic nucleotides or reactive oxygen species (ROS) are involved in integrating the different pathways (Dodd *et al.*, 2010; Newton *et al.*, 1999; Neill *et al.*, 2002). Cytokinins and ethylene are perceived by sensor hybrid histidine kinases and for cytokinins the signal is primarily transduced by a MSP (Kieber and Schaller, 2014; Merchante *et al.*, 2013). Ethylene, BRs and small polypeptides like the rapid alkalinization factor (RALF) have been shown to activate receptor kinases which transduce the signal by activating phosphorylation cascades (Wang *et al.*, 2012b; Merchante *et al.* 2013; Haruta *et al.*, 2014). In contrast, one group of the ABA receptors inactivates protein phosphatase 2C (PP2C) family proteins like ABA INSENSITIVE 1 (ABI1) and ABI2 upon ABA binding (Umezawa *et al.*, 2009; Park *et al.*, 2009; Golldack *et al.*, 2014). This in turn leads again to the activation of kinases like SUCROSE NONFERMENTING 1-RELATED PROTEIN KINASEs 2 (SnRK2s) and thereby to phosphorylation cascades (Vlad *et al.*, 2009). Auxins, GAs, JA, SA and SLs are bound by receptors which are part of E3 ligases and which target transcriptional repressors of gene expression like auxin/indole-3-acetic acid (AUX/IAA) and DELLA proteins for degradation upon hormone binding (Tan *et al.*, 2007; Park *et al.*, 2013; Fu *et al.*, 2012; Seto and Yamaguchi, 2014; Smith and Li, 2014).

## 1.1 The ubiquitin-26S proteasome system and E3 ligases

The ubiquitin-26S proteasome system starts with adenosine triphosphate (ATP) dependent activation of ubiquitin by the ubiquitin-activating enzyme (E1) (Hu and Vierstra, 2011). Then, the activated ubiquitin is transferred to the ubiquitin-conjugating enzyme (E2) which in turn interacts with the ubiquitin ligase (E3 ligase) which ubiquitinates the specific target protein (Hu and Vierstra, 2011). The specificity of ubiquitination is achieved by the E3 ligases as it is suggested that more than 1500 different E3 ligases occur in *Arabidopsis thaliana* (Gagne *et al.*, 2002; Lee *et al.*, 2008; Mudgil *et al.*, 2004; Stone *et al.*, 2005; Hua and Vierstra, 2011). One important group of E3 ligases are the CULLIN-RING ligases (CRLs) (Stone *et al.*, 2005; Hua and Vierstra, 2011). They share a common backbone including the CULLIN (CUL) scaffold protein, the REALLY INTERESTING NEW GENE DOMAIN (RING)-containing protein RING-BOX 1 (RBX1) and a substrate recognition subunit (Petroski *et al.*, 2005; Bosu *et al.*, 2008; Hua and Vierstra, 2011). According to the nature of their substrate and their associated CUL five major types can be distinguished for the CRLs whereas just three of them have been identified in plants (Petroski *et al.*, 2005; Bosu *et al.*, 2008; Hua and Vierstra, 2011). These are SCF CRLs, BTB CRLs and DWD CRLs (Hua and Vierstra, 2011). SCF CRLs are named according to their subunits S-PHASE KINASE-ASSOCIATED PROTEIN 1 (SKP1), CUL1 and an F-box protein which works as substrate recognition subunit (Gagne *et al.*, 2002; Hua and Vierstra, 2011; Yu *et al.*, 2015). BTB CRLs comprise CUL3, RBX1 and members of the BROAD COMPLEX/TRAMRACK/BRICK-A-BRACK (BTB) family (Gingerich *et al.*, 2005). DWD CRLs are composed of CUL4, RBX1 and substrate recognition subunit consisting of DAMAGED DNA BINDING 1 (DDB1) and a set of DDB1-BINDING/WD-40 DOMAIN CONTAINING (DWD) proteins which for instance comprises CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) which is involved in light signaling (Hua and Vierstra, 2011; Huang *et al.*, 2014)

## 1.2 The multistep phosphorelay (MSP) system

The MSP system appears to have evolved from the bacterial two-component signaling (TCS) system (Pekárová *et al.*, 2016). The bacterial TCS system comprises two conserved proteins: the histidine kinase which perceives a certain signal and the response regulator (Appleby *et al.*, 1996; Pekárová *et al.*, 2016). Upon signal perception the histidine kinase is activated and autophosphorylation occurs at a conserved His residue in the histidine kinase domain (West and Stock, 2001; Skerker *et al.*, 2008; Pekárová *et al.*, 2016). Subsequently the phosphate group is transferred to a conserved Asp residue in the receiver domain of the response regulator (West ad Stock, 2001; Skerker *et a.*, 2008; Pekárová *et al.*, 2016). Thereby, the response regulator is activated and mediates the final response by interaction with gene or protein targets (Casino *et al.*, 2009; Pekárová *et al.*, 2016). Dephosphorylation of the response regulator in turn leads to its inactivation and attenuation of TCS (Casino *et al.*, 2009; Pekárová *et al.*, 2016).

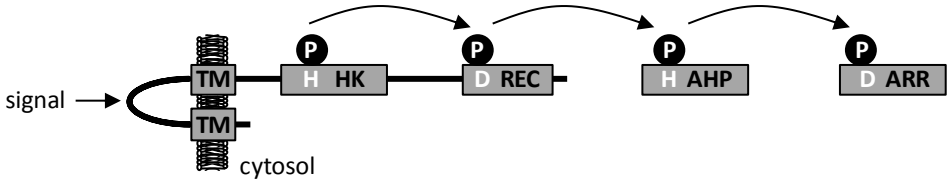



figure 1.1: The multistep phosphorelay system
Sensor hybrid histidine kinases which comprise transmembrane domains (TMs), a histidine kinase domain (HK) with a conserved histidine residue (white H) and a receiver domain with a conserved aspartate residue (white D) are mainly localized to membranes. Upon signal perception in the exracytosolic domain autophosphorylation occurs at the cytoplasmic histidine kinase domain. The phosphate group (white P) is subsequently intramolecularly transferred to the conserved aspartate in the receiver domain, then transferred to the conserved histidine residue of the AHP and subsequently to the conserved aspartate residue in the response regulator which leads to its activation and mediation of gene expression.

The MSP system in A*rabidopsis thaliana* comprises three components (fig. 1.1): The sensor hybrid histidine kinase (AHK) which comprises the histidine kinase domain with the conserved His residue and a receiver domain with a conserved Asp residue, the phosphotransfer protein (AHP) with a conserved His residue and the response regulator (ARR) with a conserved Asp residue (Stock *et al.*, 2000; West and Stock, 2001; Lohrmann and Harter, 2002; Oka *et al.*, 2002; Horak *et al.* 2011). Upon signal perception, autophosphorylation of the conserved His residue of the AHK takes place (Miwa *et al.*, 2007; Hothorn *et al.*, 2011b). Subsequently the phosphate group is intramolecularly transferred from the His residue in the kinase domain to the Asp residue in the receiver domain and from there to the His residue of the AHP (Gruhn *et al.*, 2014; Lohrmann and Harter, 2002; Oka *et al.*, 2002). AHPs shuttle continuously between the cytoplasm and the nucleus (Gruhn *et al.*, 2014; Punwani *et al.*, 2010). From the AHP the phosphate group is transferred to the conserved Asp residue in the receiver domain of the ARR (Lohrmann and Harter, 2002; Oka *et al.*, 2002). The activated ARR mediates the final response by interaction with gene or protein targets (Lohrmann and Harter, 2002; Oka *et al.*, 2002; Pekárová *et al.*, 2016).

In *Arabidopsis thaliana* there are eleven AHKs which can be divided into different groups: the five ethylene receptors, the three cytokinin receptors and the AHKs whose signal has so far not yet been

identified although AHK5 is assumed to be a redoxsensor and AHK1 to be an osmosensor (Kumar *et al.*, 2013; Pekárová *et al.*, 2016). CYTOKININ INSENSITIVE 1 (CKI1) was at first assumed to be involved in cytokinin signaling as well until it was shown that CKI1 does not bind cytokinin due to a missing cytokinin-binding domain (Yamada *et al.* 2001; Pekárová *et al.*, 2016). Furthermore, there are five *Arabidopsis thaliana* phosphotransfer proteins (AHPs) and one pseudo-AHP, AHP6, which act partially redundant as positive regulators in cytokinin signaling (Kumar *et al.*, 2013; Hutchison *et al.* 2006). For the mediation of the final response, there are 23 A*rabidopsis thaliana* response regulators (ARRs) (Müller and Sheen, 2007). The ARRs can be further divided into three subgroups: Type-A ARRs, type-B ARRs and type-C ARRs (Mason *et al.*, 2004; Müller and Sheen, 2007; Schaller *et al.*, 2007; To and Kieber, 2008).

Type-A ARRs comprise ARR3, ARR4, ARR5, ARR6, ARR7, ARR8, ARR9, ARR15, ARR16 and ARR17 (Müller and Sheen, 2007). They consist of the receiver (REC) domain with the conserved Asp residue which is involved in the phosphorelay and a short carboxy terminal domain (To and Kieber, 2008). The carboxy terminal domain is involved in mediating interactions with proteins to regulate their activity (D'Agostino *et al.*, 2000). Type-A ARRs localize in the nucleus as well as in the cytoplasm and act partially redundant (Sweere *et al.*, 2001; Dortay *et al.*, 2008) The expression of type-A ARRs is fastly upregulated after application of cytokinin in a type-B ARR-dependent manner which enables the negative feedback regulation of the cytokinin signaling pathway (Caesar, 2010; D'Agostino *et al.* 2000; Pekárová *et al.*, 2016).

Type-B ARRs comprise ARR1, ARR2, ARR10, ARR11, ARR12, ARR13, ARR14, ARR18, ARR19, ARR20 and ARR21, act as nuclear localized transcription factors and consist of the REC domain, a Myb-like DNA binding domain, the GARP domain and a transactivation domain (Mason *et al.*, 2004; Imamura *et al.*, 1999; Riechmann *et al.*, 2000; Grefen and Harter, 2004; Lohrmann *et al.*, 2001; Hosoda *et al.*, 2002; Müller and Sheen, 2007; Argyros *et al.* 2008). Like type-A ARRs also type-B ARRs can act partially redundant (Sakai *et al.*, 2001).

Type-C ARRs comprise ARR22 and ARR24 (Kiba *et al.*, 2004). They miss an output domain but at least ARR22 has been shown to interact with components of the MSP system (Horak *et al.*, 2008).

## 1.3 Cytokinins

Cytokinins are $N^6$-substituted adenine derivatives and they play major roles in apical dominance, lateral root, vascular and gametophyte development, leaf senescence, phyllotaxis, sink/source relationships, nutrient uptake as well as cell cycle control, circadian rhythm and plant defense (Kieber and Schaller, 2014). They bind to the CHASE (extracytosolic cyclases/hisidine kinases associated sensor extracellular)-domain of AHK2, AHK3 and AHK4 which are localized in the membrane of the endoplasmic reticulum (ER) (Anantharaman, 2001; Heyl *et al.*, 2007; Caesar *et al.*, 2011b; Wulfetange *et al.*, 2011; Kieber and Schaller, 2014). After cytokinin perception autophosphorylation of the AHKs occurs and a MSP transports the signal to the nucleus to adjust gene expression (Kieber and Schaller, 2014; Pekárová *et al.*, 2016).

## 1.4 Brassinosteroids

Brassinosteroids regulate numerous processes like seed germination, stomata development, vascular differentiation, plant architecture, flowering, male fertility, senescence, photomorphogenesis and innate immunity (Wang *et al.*, 2012b). Furthermore they have major effects on cell expansion (Mandava, 1988). Extensive studies of the brassinosteroid signaling pathway revealed major components and assembled them into a signal transduction pathway (Wang *et al.*, 2012b). Brassinosteroids (BR) bind to the leucine-rich repeat receptor kinase (LRR-RK) BRASSINOSTEROID-INSENSITIVE 1 (BRI1) which activates BRI1 kinase activity (Wang *et al.*, 2012b; Li and Chory, 1997; Kinoshita *et al.*, 2005; Hothorn *et al.*, 2011a; She *et al.*, 2011; Wang *et al.*, 2001). Upon activation of kinase activity, BRI1 dissociates from BRI1 KINASE INHIBITOR 1 (BIK1) and associates with the coreceptor kinase BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) (Jaillais *et al.*, 2011; Wang and Chory, 2006; Wang *et al.*, 2008; Li *et al.*, 2002b; Nam *et al.*, 2002). BAK1 interacts not exclusively with BRI1 but with several plasma membrane-localized receptor kinases including the LRR-RK FLAGELLIN-SENSITIVE 2 (FLS2) which perceives the pathogen associated molecular pattern (PAMP) flagellin22 (flg22) and therefore plays an eminent role not just in BR signaling but also in plant innate immunity and other signal transduction pathways (Sun *et al.* 2013; Wang *et al.*, 2014a; Ladwig *et al.*, 2015). Association of BR-activated BRI1 with BAK1 leads to sequential transphosphorylation of BRI1 and BAK1 (Wang *et al.*, 2008). Furthermore, activated BRI1 phosphorylates BR-SIGNALING KINASE (BSKs) and CONSTITUTIVE DIFFERENTIAL GROWTH 1 (CDG1) families of kinases (Wang *et al.*, 2012b; Tang *et al.*, 2008; Kim *et al.*, 2011). BSKs and CDG1 interact and activate the Ser/Thr protein phosphatase BRI1-SUPPRESSOR 1 (BSU1) which in turn dephosphorylates and inactivates the glycogen synthase kinase 3 (GSK3)-like kinase BRASSINOSTEROID-INSENSITIVE 2 (BIN2) (Wang *et al.*, 2012b; Kim *et al.*, 2009; Kim *et al.*, 2011; Lau and Deng, 2012; Li *et al.*, 2002a). The phosphorylated and active kinase BIN2 phosphorylates the two homologous transcription factors BRASSINAZOLE-RESISTANT 1 (BZR1) and BRI1-EMS-SUPPRESSOR 1 (BES1) which is also named BZR2 (He *et al.*, 2002; Wang *et al*, 2002; Yin *et al*. 2002). The phosphorylation of BZR1 and BES1 leads to their inactivation of transcriptional regulation through cytoplasmic retention with the help of 14-3-3 proteins as well as through their targeting for degradation by an E3-ligase (He *et al.*, 2002; Wang *et al.*, 2002; Yin *et al.*, 2002; Gampala *et al.*, 2007). In addition to BIN2, BZR1 can be phosphorylated by MPK4 and BES1 by MPK6 (Asai *et al.*, 2002; Wang *et al.*, 2013; Kang *et al.*, 2015b). Upon cytoplasmic retention BZR1 and BES1 can be dephosphorylated by a cytoplasmic protein phosphatase 2A (PP2A) and be relocated to the nucleus (Tang *et al.*, 2011; Farkas *et al.*2007). BZR1 and BES1 act as TFs in cooperation with other TFs like the basic helix-loop-helix (bHLH) TFs PHYTOCHROME INTERACTING FACTORS (PIFs) or with Groucho/TUP1-like transcriptional corepressors like TOPLESS (TPL) and TOPLESS-RELATED (TPR) proteins (Gampala *et al.*, 2007; Causier *et al.*, 2012; Oh *et al.*, 2012; Oh *et al.*, 2014). The interaction of BZR1 and TPL for instance leads to the repression of the GATA family TF *GATA4* and the B-box zinc finger family protein *BZS1* which act as positive regulators on photomorphogenesis whereas the interaction of BES1 with TPL represses *ABI3* expression (Luo *et al.*, 2010; Fan *et al.*, 2012; Oh *et al.*, 2014; Ryu *et al.*, 2014). Beside phosphorylation of BSU1, CDG1 also phosphorylates the phosphatases BRI1 SUPRESSOR 1-LIKE 1 (BSL1), BSL2 and BSL3 (Kim *et al.*, 2011). BSL2 in

turn dephosphorylates the kinase BSK8 which then exhibits kinase activity towards the SUCROSE PHOSPHATE SYNTHASE 1F (SPS1F) (Wu *et al.*, 2014). The phosphorylation of SPS1F can be removed by the PP2C family protein ABSCISSIC ACID INSENSITIVE 1 (ABI1) which provides a link to abscissic acid signaling (Nishimura *et al.*, 2010).

Furthermore, the abundance of BR leads, like light-activated phyA, to the inhibition of the expression of the NO APICAL MERISTEM domain transcriptional regulator superfamily protein ATAF2 (Peng *et al.*, 2015). ATAF2 inhibits the expression of *NITRILASE 2* (*NIT2*) which is involved in auxin biosynthesis and of cytochrome P450 superfamily proteins *PHYTOCHROME B ACTIVATION TAGGED SUPPRESSOR 1* (*BAS1*) and *SUPPRESSOR OF PHYB-4 7* (*SOB7*) which are involved in the degradation of BR (Huh *et al.*, 2012; Bartling *et al.*, 1992; Bartling *et al.* 1994; Turk *et al.*, 2005; Peng *et al.*, 2015). The signaling of BRs provides cross-talk with at least IAA-, ABA-, GA- and light-signaling.

## 1.5 Auxins

Auxins play a critical role in apical dominance, the differentiation of the vascular tissue, lateral root development and tropic responses to abiotic stimuli as well as cell extension, cell division and cell differentiation (Li *et al.*, 2016b; Guilfoyle and Hagen, 2007; Mockaitis and Estelle, 2008; Su *et al.*, 2014; Went and Thimann, 1937). It is assumed that auxin signaling occurs due to acting as “molecular glue” that promotes the interaction between the auxin receptor TRANSPORT INHIBITOR RESPONSE 1 (TIR1) and auxin/indole-3-acetic acid (AUX/IAA) proteins (Tan *et al.*, 2007; Ruegger *et al.*, 1998). TIR1 is a F-box component and thereby the substrate recognition subunit of the SCF complex which is a SCF CRL E3 ligase (Ruegger *et al.*, 1998; Smalle and Vierstra, 2004; Hua and Vierstra, 2011; Yu *et al.*, 2015). Therefore binding of auxin to TIR1 leads to the recruitment of AUX/IAA proteins to the SCF complex and to their targeting for degradation (Ruegger *et al.*, 1998; Dharmasiri *et al.* 2005; Kepinski and Leyser, 2005; Tan *et al.*, 2007). AUX/IAA proteins have been shown to act as transcriptional repressors by interacting with and thereby inhibiting the transcription activating function of AUXIN RESPONSE FACTORs (ARFs) (Tiwari *et al.*, 2001; Hagen and Guilfoyle, 2002; Hellmann and Estelle, 2002, Tiwari *et al.*, 2003; Tatemasu *et al.*, 2004). In *Arabidopsis thaliana* there are 29 *AUX/IAA* genes and 22 *ARF* genes (Liscum and Reed, 2002; Guilfoyle and Hagen, 2007). Most AUX/IAA proteins comprise four conserved domains: Domain I which is an amino terminal repression domain, domain II which comprises a recognition sequence for the SCF complex and thereby acts as regulatory domain of protein stability, domain III and domain IV which both contribute to protein interactions with other AUX/IAA proteins and ARFs (Tatemasu *et al.*, 2004; Korasick *et al.*, 2014). ARFs reveal three conserved domains: an amino terminal B3-type DNA-binding domain which is important for the regulation of gene expression and two carboxy terminal domains which share homology with domain III and domain IV of AUX/IAA proteins and therefore confer protein-interaction with AUX/IAAs and other ARFs (Tiwari *et al.*, 2003; Tatemasu *et al.*, 2004; Korasick *et al.*, 2014).

One of the largest family of auxin-induced genes is the family of *SMALL AUXIN UP-RNA* (*SAUR*) genes which comprises 79 members in A*rabidopsis thaliana* (Hagen and Guilfoyle, 2002; Spartz *et al.*, 2014). Thereby, the *SAUR19* and *SAUR63* subfamilies have been suggested to work as positive effectors of cell expansion (Franklin *et al.*, 2011; Chae *et al.*, 2012, Spartz *et al.*, 2012; Spartz *et al.*, 2014). For SAUR19 it has been shown, that it interacts with and inhibits the activity of the subfamily D

of PP2C family proteins which in turn regulate the activity of plasma membrane localized $H^+$-ATPases (AHAs) (Spartz *et al.*, 2014). AHA-activity is regulated by diverse signaling pathways which all result in differential phosphorylation of the regulatory carboxy terminus of AHAs especially of the penultimate Thr-residue (Kinoshita and Shimazaki, 1999; Kinoshita and Shimazaki, 2002; Hayashi *et al.*, 2010; Wang *et al*, 2014). Phosphorylation of the regulatory carboxy terminus leads to the association with 14-3-3 proteins which are involved in the regulation of AHA activity as well (Fuglsang *et al.*, 1999). AHAs work as proton pumps and are therefore involved in the regulation of apoplastic pH which plays major roles in the acid growth theory (Rayle and Cleland, 1992; Kinoshita and Shimazaki, 1999; Hager, 2003).

## 1.6 Abscissic acid

Abscissic acid (ABA) is involved in the regulation of various aspects of plant growth and development including embryo maturation, seed dormancy, seed germination, cell division and elongation, floral induction, nutrient signaling, turgor maintenance, stomatal regulation, senescence and responses to environmental stresses like osmotic stress, pathogen attack and UV radiation (Finkelstein, 2013). To date, three classes of ABA receptors have been identified (Finkelstein, 2013). First, there are the membrane localized ABA-binding G PROTEIN-COUPLED RECEPTOR-TYPE G PROTEIN 1 (GTG1) and GTG2 which are assumed to contribute to fertility, hypocotyl and root growth and responses to light and sugars although *gtg1 gtg2* knock down mutants respond normally in classic ABA responses (Pandey *et al.*, 2009; Jaffé *et al.*, 2012; Finkelstein, 2013; Golldack *et al.*, 2014). Second, there is evidence for ABA perception by the chloroplast-localized H subunit of $Mg^{2+}$-chelatase which is involved in plastid-to-nucleus retrograde signaling and mediates ABA signaling as a positive regulator in seed germination, post-germination growth and stomatal movement (Shen *et al.*, 2006; Finkelstein, 2013; Golldack *et al.*, 2014). The to date best characterized ABA receptors are the nucleocytoplasmic receptors PYRABACTIN RESISTANCE / PYRABACTIN RESISTANCE-LIKE / REGULATORY COMPONENT OF ABA RECEPTORS (PYR/PYL/RCARs) which inhibit protein phosphatase 2C (PP2C) family proteins like ABA INSENSITIVE 1 (ABI1) and ABI2 upon ABA binding (Ma *et al.*, 2009; Park *et al.*, 2009; Finkelstein, 2013; Golldack *et al.*, 2014). Additional members of this clade of PP2Cs have been shown to contribute to ABA and stress signaling and their knock down mutants either reveal a hypersensitive phenotype to ABA or no phenotype which is then due to redundancy (Kuhn *et al.*, 2006; Nishimura *et al.*, 2007; Finkelstein, 2013). Inactivation of these PP2Cs leads to the accumulation of active SnRK2s which regulate ABA-responsive transcription factors including ABA-responsive promoter element binding factors by phosphorylation (Ma *et al.*, 2009; Park *et al.*, 2009; Umezawa *et al.*, 2009; Vlad *et al.*, 2009; Golldack *et al.*, 2014). The phosphorylation of these transcription factors leads to the activation of ABA-responsive genes and therefore to the activation of ABA-responsive physiological processes (Umezawa *et al.*, 2009; Vlad *et al.*, 2009; Golldack *et al.*, 2014). Important transcription factors in this pathway comprise ABSCISSIC ACID INSENSITIVE 3 (ABI3), ABI4 and ABI5 (Koornneef *et al.*, 1984). ABI3 belongs to the B3 transcription factor family, ABI4 to the APETALA2 (AP2) family and ABI5 to the basic leucine zipper (bZIP) family (Giraudat *et al.*, 1992; Finkelstein, 1994; Finkelstein *et al.*, 1998).

ABI3, ABI4 and ABI5 can be phosphorylated by SnRK2s as well as by BIN2 which provides a link to BR signaling (Lynch *et al.*, 2012; Yuan *et al.*, 2013; Hu and Yu, 2014). This phosphorylation can be removed by PHYTOCHROME-ASSOCIATED PROTEIN PHOSPHATASE 1 (FyPP1) and FyPP3 which are known to also dephosphorylate and thereby regulate the auxin efflux carriers PIN-FORMED 1 (PIN1), PIN3 and PIN4 and thereby provide a link to light and auxin signaling (Dai *et al.*, 2012; Dai *et al.*, 2013; Yuan *et al.*, 2013). Furthermore, type-A ARRs interact with ABI3, ABI4 and ABI5 at least by the regulation of their expression and thereby provide a link between MSP, cytokinin and ABA signaling (Wang *et al.*, 2011a). Further cross-talk is achieved by the dependence of ABA signaling on second messengers (Finkelstein, 2013).

## 1.7 Gibberellic acids

Gibberellic acids (GAs) belong to a large family of tetracyclic diterpenoids and are plant hormones which regulate various transition processes like seed germination and flowering (Sun and Gubler, 2004; Park *et al.*, 2013; Xu *et al.*, 2014b). Furthermore they are involved in stem and hypocotyl elongation, leaf expansion and responses to environmental stresses (Gao *et al.*, 2011; Park *et al.*, 2013; Xu *et al.*, 2014b). GA binding to the soluble receptor GA-INSENSITIVE DWARF 1 (GID1) binds leads to GID1 association with the amino terminus of the GRAS (GA INSENSITIVE; REPRESSOR OF *ga1-3*; SCARECROW) subgroup of DELLA proteins (Murase *et al.*, 2008; Shimada *et al.*, 2008; Golldack *et al.*, 2014). This enables the interaction of DELLAs with the SCF E3 ligase which targets the DELLA protein for 26S proteasomal degradation (McGinnis *et al.*, 2003; Sasaki *et al.*; 2003). The destabilization of DELLA proteins leads to the release of TFs like PIFs, BZR1 and the AUXIN RESPONSE FACTOR 6 (ARF6) (Choi and Oh, 2016; de Lucas *et al.*, 2008; Feng *et al.*; 2008; Bai *et al.*, 2012b; Oh *et al.*, 2014). DELLA proteins have been shown to integrate diverse signaling pathways including IAA-, BR-, JA-, ethylene- and light- as well as temperature- and osmotic stress signaling (Xu *et al.*, 2014b).

## 1.8 Ethylene

The gaseous hormone ethylene is involved in the regulation of germination, fruit ripening, senescence and stress responses (Bleecker and Kende, 2000; Liu *et al.*, 2010; Merchante *et al.*, 2013; Pekárová *et al.*, 2016). Ethylene is perceived by ETHYLENE RESPONSE 1 (ETR1), ETR2, ETHYLENE RESPONSE SENOR 1 (ERS1), ERS2 and ETHYLENE INSENSITIVE 4 (EIN4) which are similar to the histidine kinase proteins (Bleecker *et al.*, 1988; Chang *et al.* 1993; Hua *et al.*; 1998; Sakai *et al.*, 1998; Liu *et al.*, 2010). Thereby, ETR1 and ERS1 comprise a histidine kinase domain with the signature motifs essential for histidine kinase activity whereas in ETR2, ERS2 and EIN4 some consensus amino acid residues which are essential for histidine kinase activity are lacking (Liu *et al.*, 2010).Upon ethylene perception the Raf-like Ser/Thr kinase CONSTITUTIVE TRIPLE RESPONSE (CTR1) is inactivated which leads to the phosphorylation of EIN2. By an unknown mechanism the carboxy terminus of EIN2 is cleaved off and translocated to the nucleus where it induces the degradation of EIN3-BINDING F-BOX PROTEIN 1 and EBF2 but stabilizes EIN3 and ETHYLENE-INSENSITIVE 3-LIKE 1 which activate ethylene target genes (Ju *et al.*, 2012; Ji and Guo, 2013; Solano *et al.* 1998; Merchante *et al.*, 2013).

## 1.9 Jasmonates

Jasmonates (JAs) are lipid-derived compounds and involved in the response to herbivory, mycorrhiza and the regulation of plant immunity as well as in the regulation of seed germination, seedling development, root growth, flower and seed development, tuber formation and senescence (Wasternack, 2007; Wasternack and Hause, 2013). They are perceived by negative regulators of JA-induced gene expression, the JASMONATE-ZIM DOMAIN proteins which are upon JA binding targeted for degradation by the SCF E3 ligase with CORONATIVE INSENSITIVE 1 as substrate recognition subunit for the SCF complex (Chini *et al.*, 2007; Thines *et al.*, 2007; Yan *et al.*, 2007; Xie *et al.*, 1998; Wasternack and Hause, 2013; Hua and Vierstra, 2011).

## 1.10 Salicylic acid

Salicylic acid (SA) is a phenolic compound which has been shown to activate plant defense responses especially systemic acquired resistance (SAR) as well as to play a role in plant responses to abiotic stresses like drought, chilling, heat, osmotic stress and heavy metal toxicity (Rivas-San Vicente and Plasencia, 2011; Kumar, 2014). SA binds to its receptors NPR1-like protein 3 (NPR3) and NPR4 which are CUL3 E3 ligase substrate recognition subunits for NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1) degradation (Fu *et al.*, 2012). This leads to SA mediated signal transduction (Fu *et al.*, 2012; Kumar, 2014).

## 1.11 Strigolactones

Strigolactones (SLs) have been shown to regulate root and shoot development and can promote symbiotic relationships with mycorrhizal fungi and nitrogen-fixing bacteria (Smith and Li, 2014). It is assumed that SL perception and signal transduction occurs similar to GAs, JAs and auxins which depends on recognition of SL by a substrate recognition subunit of the SCF CRL E3 ligase and which targets proteins for degradation (Seto and Yamaguchi, 2014; Ishikawa *et al.*, 2005; Stirnberg *et al.* 2002; Johnson *et al.*, 2006). Evidence has been provided for cross-talk of SL-signaling with GA- and BR-signaling (Smith and Li, 2014).

## 1.12 Light signaling

The abiotic factor light highly influences plant growth and development as light provides energy for carbon fixation through photosynthesis as well as information for the adaption to the different aspects of light like light intensity and light quality which comprises the spectral composition and spatiotemporal patterns (Menon *et al.*, 2016). For the sensing of and for the adaption to these aspects of light, plants have several classes of photoreceptors which have specific as well as overlapping functions (Menon *et al.*, 2016). The acclimation of plants to UV-B light is mediated by the UV-B light receptor UVB-RESISTANCE 8 (Favory *et al.*, 2009; Menon *et al.*, 2016). Blue light is perceived by PHOTOTROPIN 1 (PHOT1) and PHOT2, by photoreceptors of the ZEITLUPE (ZTL)-family as well as by CRYPTOCHROME 1 (CRY1) and CRY2 (Chaves *et al.*¸ 2011; Ito *et al.*, 2012; Christie *et al.*, 2015; Fankhauser and Christie, 2015; Menon *et al.* 2016). Phototropins play major roles in the regulation of light responses which are important to optimize photosynthesis which includes phototropism, chloroplast and stomatal movement (Chen *et al.*, 2004; Menon *et al.*, 2016). They sense light with their

amino terminal photosensory domain in which a flavin mononucleotide (FMN) molecule is bound to the LIGHT, OXYGEN, VOLTAGE (LOV) domain which allows light sensing and which is structurally closely related to Per-Arnt-Sim (PAS) domains (Briggs and Christie, 2002; Chen *et al.*, 2004). Furthermore, phototropins comprise a carboxy terminal Ser/Thr protein kinase domain which leads to light-regulated autophosphorylation as initial step of phototropin dependent signaling (Briggs and Christie, 2002; Chen *et al.*, 2004). Upon phosphorylation, phototropins are bound by 14-3-3 proteins (Kinoshita *et al.*, 2003; Chen *et al.*, 2004). Like phototropins, ZTL, LOV KELCH REPEAT PROTEIN 2 and FLAVIN-BINDING KELCH REPEAT F-BOX 1 comprise a LOV-domain which binds a FMN molecule for light perception with the difference of a very slow dark-reversion rate compared to the phototropins (Christie *et al.*, 2015; Chen *et al.* 2004). Photoreceptors of the ZTL-family are involved in maintenance of circadian clock function and photoperiod-dependent induction of flowering (Imaizumi *et al.*, 2003; Yanovsky and Kay, 2003; Chen *et al.*, 2004). Beside blue light, CRY1 and CRY2 are known to perceive UV-A light (Lin, 2002; Liscum *et al.*, 2003; Chen *et al.*, 2004). They are involved in the regulation of inhibition of hypocotyl growth, promotion of leaf expansion as well as in chlorophyll and anthocyanin synthesis during seedling de-etiolation (Lin, 2002; Liscum *et al.*, 2003; Chen *et al.*, 2004). Furthermore they contribute to photoperiod-dependent induction of flowering and to resetting of the circadian oscillator (Yanovsky and Kay, 2003; Cashmore, 2003; Chen *et al.* 2004). Photosensing by CRY1 and CRY2 works through the amino terminal photolyase homology region which noncovalently binds a catalytic flavin adenine dinucleotide (FAD) and a pterin or deazaflavin as light-harvesting chromophore (Lin *et al.*, 1995; Sancar, 2003; Chen *et al.* 2004). Cryptochromes act in coordination with the red light sensing phytochromes (Chen *et al.*, 2004). In A*rabidopsis thaliana* there are five members of phytochromes: The light-labile phytochrome A (phyA) and phyB, phyC, phyD and phyE which are more or less stable in light and darkness (Chen *et al.*, 2004; Menon *et al.*, 2016). They are composed of the amino terminal photosensory domain with a covalently attached phytochromobilin and a carboxy terminal domain which comprises two PAS-domains and a histidine kinase-related domain (Quail, 1997; Yeh and Lagarias, 1998; Bolle *et al.* 2000; Kohchi *et al.*; 2001; Chen *et al.*, 2004). Still, higher plant phytochromes are Ser/Thr kinases (Yeh and Lagarias, 1998; Fankhauser, 2000; Chen *et al.*, 2004). Phytochromes are synthesized in the inactive red-light absorbing Pr-form (Phee *et al.*, 2008). Upon red-light absorption the Pr-form is converted to the active and far red-light absorbing Pfr-form (Chen *et al.*, 2004). The Pfr-form can be re-converted to the Pr-form either by the absorption of far red light or thermally which is then called dark reversion (Kendrick and Kronenberg, 1994; Chen *et al.*, 2004). In the dark, phytochromes accumulate in the cytoplasm but upon light perception they are translocated to the nucleus where they interact with and thereby regulate diverse transcription factors like PIFs, ELONGATED HYPOCOTYL 5 (HY5) or FAR-RED ELONGATED HYPOCOTYL 3 (FHY3) (Kircher *et al.*, 2002; Chen *et al.*, 2004; Hardtke *et al.*, 2000; Rolauffs *et al.*, 2012; Menon *et al.*, 2016). Phytochromes are together with cryptochromes involved in seedling development and floral induction but solely control seed germination and shade-avoidance response (Casal and Sanchez, 1998; Neff *et al.*¸ 2000; Chen *et al.*, 2004).

The absence of light, especially after germination when the seed might be buried in soil effects a developmental strategy called skotomorphogenesis or etiolated growth (Leivar *et al.*, 2008). The

etiolated growth is characterized by hypocotyl elongation, a closed apical hook and the absence of chlorophyll accumulation (Leivar *et al.*, 2008). Upon light perception, the transition from skotomorphogenic to photomorphogenic growth occurs (Leivar *et al.*, 2008). This transition is called de-etiolation and comprises the inhibition of hypocotyl elongation, unfolding of the apical hook, separation and expansion of the cotyledons and chlorophyll accumulation (Leivar *et al.*, 2008).

## 1.13 Temperature-induced hypocotyl elongation

Warmth induces hypocotyl elongation in light-grown seedlings (Gray *et al.*, 1998; Stavang *et al.*, 2009; Oh *et al.*, 2012; Delker *et al.*, 2014). The increase of the temperature leads to a decreased level of the transcription factor HY5 indicating an increased targeting of HY5 to degradation by the DET1-COP1-HY5 pathway (Delker *et al.*, 2014). This in turn leads to an increase of PIF4 protein levels as HY5 negatively regulates *PIF4* transcription (Delker *et al.*, 2014). PIF4 mediates the expression of *YUC* genes, which are necessary for auxin biosynthesis and, together with BZR1, activates the transcription of auxin responsive genes like *SAUR19* to *SAUR24* and *IAA19* and *IAA29* which results in auxin response and therefore in elongation growth although in light, PIF4 is phosphorylated by active phytochromes in the Pfr-from and thereby inhibited to bind to target promoters and targeted for degradation (Shen *et al.*, 2005; Cheng *et al.*, 2006; Oh *et al.*, 2006; Al-Sady *et al.*, 2006; Stepanova *et al.*, 2011; Won *et al.*, 2011; Oh *et al.*, 2012; Park *et al.*, 2012; Delker *et al.*, 2014).

## 1.14 Acid growth theory, cell walls and growth

The turgor-driven cell expansion requires a balance between cell wall relaxation and cell wall stiffening (Wolf *et al.*, 2012). Wall hydration, turgor-driven wall extension followed by the cross-linking of newly synthesized cell-wall components are thereby the major steps of turgor-driven cell expansion (Wolf *et al.*, 2012). Corresponding to the acid growth theory wall hydration is in part mediated by the IAA- and BR-induced activation of AHAs which cause the hyperpolarization of the plasma membrane and the acidification of the apoplast (Rayle and Cleland, 1992; Caesar *et al.*, 2011a; Wolf *et al.*, 2012). The acidification of the apoplast leads to cell-wall loosening in part through the activation of cell-wall-loosening enzymes like expansins which have an acidic pH optimum (Lee *et al.*, 2001; Yennawar *et al.*, 2006; Thompson, 2008; Wolf *et al.*, 2012). Increased wall-hydration and the activity of wall-loosening enzymes promote the extensibility of the cell-wall (Evered *et al.*, 2007; Wolf *et al.*, 2012). Dependent on the orientation of the cellulose microfibrils upon wall-loosening increased spacing between cellulose microfibrils occurs through turgor-driven cell expansion (Lloyd and Chan, 2008; Wolf *et al.*, 2012). Therefore the orientation of the cellulose microfibrils determines the direction of cell expansion which is the reason why reorientation of cellulose microfibrils can occur (Steen and Chadwick, 1981; Bashline *et al.*, 2014). This can be induced by ethylene which also influences the orientation of cortical microtubules (Steen and Chadwick, 1981). Cortical microtubules play a key role in the organization of the cellulose deposition as they control the insertion, trajectory and velocity of the cellulose synthesizing cellulose synthase complexes (Crowell *et al.*, 2009; Gutierrez *et al.*, 2009; Wolf *et al.*, 2012; Bashline *et al.*, 2014). The effect of ethylene on the orientation of cortical microtubules appears to be opposite to the effect of IAA, GA and BR (Shibaoka, 1993; Fujino *et al.*, 1995; Le *et al.*, 2005; Polko *et al.*, 2012; Wang *et al.*, 2012a; Bashline *et al.*, 2014).

Cell wall relaxation and cell expansion are suggested to be coupled with mechanosensing as the plasma membrane is stretched and pressed to the cell wall (Monshausen *et al.*, 2009; Monshausen and Gilroy, 2009). Mechanical stimuli result in an increase of cytosolic $Ca^{2+}$ levels which inhibit AHAs and open $H^+$-channels which in turn lead to an alkalinization of the apoplast and cytoplasmic acidification and therefore to the inhibition of cell expansion and growth (Monshausen *et al.*, 2009; Monshausen and Gilroy, 2009; Wolf *et al.*, 2012). Thereby, growth rate, cytosolic $Ca^{2+}$-levels and apoplastic pH oscillate with the same period (Monshausen *et al.*, 2009; Monshausen and Gilroy, 2009; Wolf *et al.*, 2012). It was assumed that the increase of cytosolic $Ca^{2+}$-levels depends on stretch-activated $Ca^{2+}$-channels of the MscS-like (MSL) family but recent studies propose the contribution of phosphorylation dependent plastidial $K^+$ EFFLUX ANTIPORTER (KEA) KEA1, KEA2 and KEA3 (Monshausen and Gilroy, 2009; Wilson *et al.*, 2013; Stephan *et al.*, 2016).

The inhibition of cell expansion through cytoplasmic acidification is supported by the stabilization of PLASMA MEMBRANE INTRINSIC PROTEINs (PIPs) in the closed pore conformation through the protonation of a conserved His-residue in the cytoplasmic loop D (Törnroth-Horsefield *et al.*, 2006; Frick *et al.*, 2013; Maurel *et al.*, 2015). PIPs build one of five subfamilies of higher plant aquaporins which are membrane channels that facilitate the transport of water and small neutral molecules across biological membranes (Maurel *et al.*, 2015). PIPs comprise a cytoplasmic amino terminus, six transmembrane domains which are connected through five loops and a cytoplasmic carboxy terminus (Maurel *et al.*, 2015). Thereby loop A, C and E are extracellular whereas loop B and D are cytoplasmic (Maurel *et al.*, 2015). Beside the conserved His-residue in loop D which stabilizes PIPs in their closed pore conformation there are regulatory phosphorylation sites at the PIP's carboxy terminus which influence the pore conformation as well (Maurel *et al.*, 2015). The phosphorylation of these residues has been shown to depend on $Ca^{2+}$ DEPENDENT PROTEIN KINASEs (CDPKs) and the LRR-RK SUCROSE-INDUCED RECEPTOR KINASE 1 (SIRK1; Sjövall-Larsen *et al.*, 2006; Wu *et al.*, 2013; Maurel *et al.*, 2015). Due to their involvement in facilitating water transport and transport of small neutral molecules, PIPs are involved in ROS detoxification and signaling, nutrient availability, diurnal and circadian rhythms, guard cell and leaf movements as well as in osmoregulation (Maurel *et al.*, 2015).

## 1.15 Osmotic stress

Osmotic stress can be induced by several different factors like for instance altered water availability, flooding and therefore altered oxygen availability, dissolved ion content, abundance of other osmotically active substances, atmospheric humidity, temperature, wind speed as well as solar irradiance (Stephan *et al.*, 2016). Therefore it is necessary to sense osmotic stress and integrate various signals to obtain a proper response to osmotic stress.

ABA is known to play a key role in adaptational processes to osmotic stress like stomatal closure, growth inhibition and osmoregulatory solute accumulation which comprises proline accumulation and recently, evidence has been provided for cross-talk between ABA-, GA- and JA-signaling in plant responses to drought (Verslues *et al.*, 2006; Wohlbach *et al.*, 2008; Finkelstein, 2013; Kumar *et al.* 2013; Golldack *et al.*, 2014). Furthermore it has been revealed that ROS contribute to a modified tolerance to osmotic stress (Zheng *et al.* 2013a, Golldack *et al.*, 2014). Other mechanisms which are

involved in the adaption to osmotic stress are the maintenance of cell wall development and the maintenance of integrity of cellular membranes at the plasma membrane as well as at the endomembranes (Cominelli *et al.*, 2008; Lippold *et al.*, 2009; Golldack *et al.*, 2014). The maintenance of integrity of cellular membranes upon osmotic stress is known to be influenced by changes of the monogalactosyldiacylglycerol and digalactosyldiacylglycerol contents in the chloroplast envelope and in thylakoid membranes as well as by lipid signaling which involves a change in the activity of phospholipase C and the diacylglycerol kinase (Torres-Franklin *et al.*, 2007; Darwish *et al.*, 2009; Golldack *et al.*, 2014). A link between ABA- and lipid-signaling has been shown by Lemtiri-Chlieh *et al.* (2003) who described that in response to ABA myo-inositol hexakisphosphate ($InsP_6$) levels are elevated and that $InsP_6$ inactivates the plasma membrane inward $K^+$-conductance in a cytosolic $Ca^{2+}$-dependent manner which promotes stomatal closure and which is assumed to be conferrable to osmotic stress signaling (Golldack *et al.*, 2014). Furthermore, intracellular $Ca^{2+}$-levels contribute to the maintenance and regulation of ion homeostasis during osmotic stress by activating the salt overly sensitive (SOS) pathway (Du *et al.*, 2011; Golldack *et al.*, 2014). Thereby, the Calcineurin B-like (CBL) protein calcium sensor SALT OVERLY SENSITIVE 3 (SOS3) as well as the CBL-interacting protein kinase (CIPK) SOS2 regulate the activity of the $Na^+/H^+$-antiporter SOS1 (Liu *et al.*, 1997; Halfter *et al.*, 2000; Liu *et al.*, 2000).

In regard to the perception of osmotic stress by osmosensors it has been hypothesized that this might include the sensing of cell volume, cell shape, membrane tension, turgor pressure, the pressure of the plasma membrane to the cell wall or macromolecular crowding (Kumar *et al.*, 2013; Wohlbach *et al.*, 2008; Hsiao, 1973; Burg *et al.*, 2007; Schliess *et al.*, 2007; Wood, 2011). So far the mechanism of osmotic stress perception is unclear but for instance in yeast, components of the high osmolarity glycerol (HOG) pathway which leads to osmotic adjustment have been elucidated (Reiser *et al.*, 2003; Saito and Tatebayashi, 2004; Kumar *et al.*, 2013). In the HOG pathway osmotic stress is perceived by the sensor hybrid histidine kinase SLN1 (Maeda *et al.*, 1994; Posas *et al.*, 1996). SLN1 transmits the signal through the SLN1-YPD1-SSK1 multistep phosphorelay to the redundant pairs of MKKKs SSK2 and SSK22 which in turn activate a MPK phosphorylation cascade (Maeda *et al.*, 1994; Posas *et al.*, 1996; Reiser *et al.*, 2003; Kumar *et al.*, 2013; Pekárová *et al.*; 2016). This pathway finally activates the MPK HOG1 which participates in the regulation of several osmotic stress responses like for instance the accumulation of glycerol as intracellular osmotically active substance (Reiser *et al.*, 2003; Dihazy *et al.* 2004; Kumar *et al.*, 2013). Beside SLN1, the HOG-pathway can be activated by the SHO1 branch (Reiser *et al.*, 2003). As the osmosensitive growth defect of the yeast *sln1/sho1* mutant can be complemented by the *Arabidopsis thaliana* cytokinin receptors AHK2, AHK3 and AHK4 as well as by AHK1 it was assumed that AHK1 might be the main osmosensor in plants (Urao *et al.*, 1999; Reiser *et al.*, 2003; Tran *et al.*, 2007; Wohlbach *et al.*, 2008).

## 1.16 AHK1

AHK1 is a sensor hybrid histidine kinase which comprises two transmembrane domains, an extracellular domain which might perceive a so far uncharacterized signal, a histidine kinase domain and a receiver domain (Urao *et al.*, 1999). It has been shown, that AHK1 localizes to the plasma

membrane after transient expression in *Nicotiana benthamiana* as well as to vesicle-like compartments which have not yet been fully characterized (Katharina Caesar, unpublished).

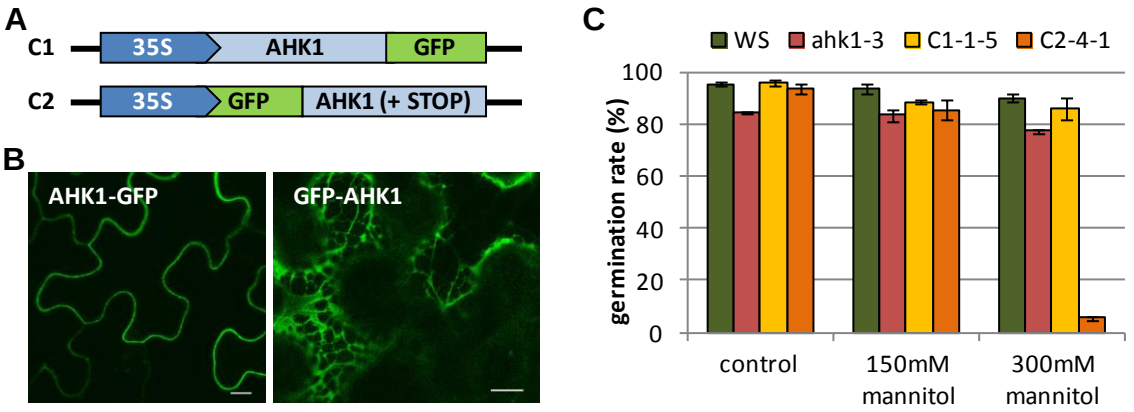



figure 1.2: AHK1 with a carboxy terminal GFP-tag complements the germination phenotype of *ahk1-3*, AHK1 with an amino terminal GFP-tag does not.
*AHK1* with a carboxy terminal *GFP*-tag (*AHK1-GFP*) under the control of the *Cauliflower Mosaic Virus (CaMV) 35S-promoter* (C1) and *AHK1* with an amino terminal *GFP*-tag (*GFP-AHK1*) under the control of the *CaMV 35S-promoter* (C2) were transiently transformed into *Nicotiana benthamiana* (B) and stably into the *ahk1* knock down mutant *ahk1-3*. The analysis with confokal microscopy (SP2) of the transient expression of AHK1-GFP and GFP-AHK1 revealed localization of AHK1-GFP at the plasma membrane and localization of GFP-AHK1 in the endoplasmic reticulum. (C) A germination assay with homozygous *Arabidopsis thaliana* lines which contained either the C1 or the C2 construct revealed that just AHK1-GFP is able to complement the germination phenotype on high mannitol stress conditions. (Unpublished data by Katharina Caesar)

The analysis of *Arabidopsis thaliana ahk1* knock down mutants revealed a contribution of AHK1 in osmoregulation (Urao *et al.*, 1999; Reiser *et al.*, 2003; Tran *et al.*; 2007; Wohlbach *et al.*, 2008; Kumar *et al.* 2013). Tran *et al.* (2007) could show, that the overexpression of AHK1 in the *Arabidopsis thaliana* Col-0 or Nos-0 ecotype enhances drought tolerance and that AHK1 in contrast to AHK2, AHK3 and AHK4 acts as positive regulator of ABA signal transduction and osmotic stress signaling. Wohlbach *et al.* (2008) revealed in the *Arabidopsis thaliana* ecotype Ws-2 that the disruption of AHK1 leads to decreased germination rates of seeds on media which are supplemented with the osmotically active substances sorbitol, mannitol, sucrose, glucose and NaCl and to decreased root elongation during growth on sorbitol supplemented media. Furthermore *ahk1* knock down mutants in the Ws-2 ecotype revealed altered ABA sensitivities in regard to decreased expression of the ABA-dependent marker gene *RESPONSIVE TO ABA 18* (*RAB18*) and the gene of *RESPONSIVE TO DESICCATION 29B* (*RD29B*) but did not show any change in stomatal aperture (Wohlbach *et al.*, 2008; Kumar *et al.*, 2013). As ABA biosynthetic genes were upregulated upon overexpression of *AHK1* an AHK1-dependent regulation of ABA synthesis was hypothesized (Wohlbach *et al.*, 2008). Tran *et al.* (2007) and Wohlbach *et al.*, (2008) concluded AHK1 to be a positive regulator of osmoregulation in the *Arabidopsis thaliana* ecotypes Nos-0, Col-0 and Ws-2. In contrast, Kumar *et al.* (2013) found, that *ahk1* knock down mutants in the Nos-0 and Col-0 ecotype revealed reduced relative water content, increased leaf water loss which is explained by an increased stomatal index, unimpaired root growth upon salt and low water potential stress as well as unimpaired ABA, proline and osmoregulatory solute accumulation which led to the conclusion of AHK1 being a negative regulator of osmoregulation. Still,

these results were obtained under unequal conditions: Beside different compositions of growth media Tran *et al.* (2007) used growth conditions of 22°C under a 16h light/8h dark cycle, Wohlbach *et al.* (2008) used 23°C under a 16h light/8h dark cycle or continuous light whereas Kumar *et al.* (2013) used 25°C under continuous light as well as 22°C-26°C under a 16h light/8h dark cycle.

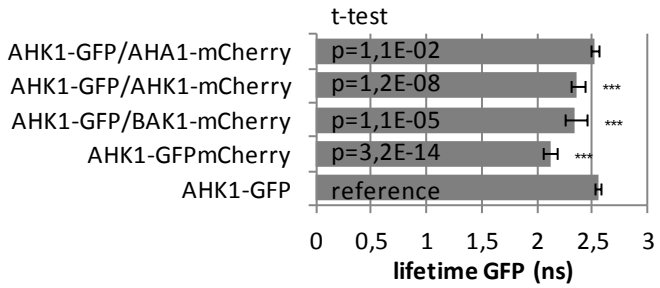



figure 1.3: FRET-FLIM suggests interaction of AHK1 and BAK1.
Transient co-expression of *AHK1-GFP* with *AHA1-mCherry*, *AHK1-mCherry* and *BAK1-mCherry* led to a drop in fluorescence lifetime for the pairs AHK1-GFP/AHK1-mCherry and AHK1-GFP/BAK1-mCherry as well as for the positive control AHK1-GFPmCherry indicating that AHK1 forms homodimers and interacts with BAK1. Stars indicate statistical significance. (Unpublished data by Katharina Caesar)

Additional studies of Katharina Caesar showed, that AHK1 with a carboxy terminal but not with an amino terminal GREEN FLUORESCENT PROTEIN (GFP)-tag could complement the germination phenotype of *ahk1-3* under osmotic stress conditions which were applied with the osmotically active substance mannitol (Katharina Caesar, unpublished; fig. 1.2). Furthermore it was revealed, that expression of mCherry with an additional nuclear localization signal (NLS) under the control of the *RD29B* promoter is enhanced upon mannitol treatment in an AHK1- and temperature-dependent manner (Katharina Caesar, unpublished). Moreover, a mating-based split-ubiquitin assay in *Saccharomyces cerevisiae* and a Förster resonance energy transfer (FRET)-fluorescence lifetime imaging (FLIM) study in *Nicotiana benthamiana* suggested the interaction of AHK1 and BAK1 (Katharina Caesar, unpublished; fig. 1.3).

## 1.17 Objective of this work

AHK1 was suggested to work as osmosensor in plants (Urao *et al.*, 1999; Reiser *et al.*, 2003; Tran *et al.*, 2007; Wohlbach *et al.*, 2008). Several studies could show, that AHK1 indeed is involved in the regulation of osmotic stress adjustment (Urao *et al.*, 1999; Reiser *et al.*, 2003; Tran *et al.*, 2007; Wohlbach *et al.*, 2008; Kumar *et al.*, 2013). Nevertheless there were opposing results whether AHK1 works as positive or negative regulator of osmoregulation (Tran *et al.*, 2007; Wohlbach *et al.*, 2008; Kumar *et al.*; 2013).

Moreover, nothing more was known about the components of the AHK1-dependent signal transduction pathway except the fact, that AHK1 is a sensor hybrid histidine kinase which contributes to the multistep phosphorelay system and that it interacts with BAK1 (Urao *et al.*, 2000; Dortay *et al.*, 2006; Dortay *et al.*, 2008; Katharina Caesar, unpublished).

The aim of this work was, to clarify whether AHK1 acts as positive or negative regulator on osmoregulation, to gain insight into the molecular mechanisms of the signal transduction pathway and its components and to investigate whether AHK1 acts as mechano-sensitive osmosensor.

To clarify whether AHK1 acts as positive or negative regulator on osmoregulation a consistent phenotype for all *ahk1* knock down mutants in all three previously described *Arabidopsis thaliana* ecotypes should be found (Tran *et al*. 2007; Wohlbach *et al.*, 2008; Kumar *et al.* 2013). To support the finding of the respective result it is important to gain insight into the molecular mechanisms of signal transduction. As it was previously suggested that the osmoregulation in *Arabidopsis thaliana* works similar to the HOG pathway in yeast with AHK1 as osmosensor a transition from the His-to-Asp multistep phosphorelay to Ser/Thr/Tyr phosphorylation was hypothesized (Reiser *et al.*, 2003; Tran *et al*. 2007; Wohlbach *et al.*, 2008). The comparative analysis of the phosphoproteome of an *ahk1* knock down mutant and the wildtype after treatment with mock or the osmotically active substance mannitol was suggested to provide the prove of a possible transition. This should comparatively be investigated as well for *ahk2 ahk3* knock down mutants and their wildtype Col-0 which were treated with mock or kinetin. Although it would have been interesting to identify a similar pathway like in yeast, it should be tested whether interactions of AHK1 with other plasma membrane localized proteins are involved in osmotic stress signaling. Therefore AHK1-dependent and mannitol-dependent differentially phosphorylated proteins which were quantified in the phosphoproteome should be tested on direct protein-protein interaction with AHK1.

To investigate whether AHK1 acts as mechano-sensitive osmosensor the evolutionary conservation as well as the structure of the extracellular domain of AHK1 should be analyzed and determined. This should occur with the help of sequence analysis and sequence alignments as well as through the cloning, expression and crystallization of the extracellular domain of AHK1.

# 2 MATERIAL

## 2.1 Organisms

### 2.1.1 Escherichia coli strains

table 2.1: Escherichia coli strains

| strain (company) | genotype | function |
|---|---|---|
| NEB®5α (New England Biolabs) | *fhuA2 Δ(argF-lacZ)U169 phoA glnV44 Φ80 Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17* | Used for cloning and amplification of vectors |
| One Shot®TOP10 (invitrogen) | *F- mcrA Δ(mrr-hsdRMS-mcrBC) Φ80ΔlacM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu)7697 galE15 galK16 rpsL (StrR) endA1 λ-* | Used for cloning of *Entry* vectors with TOPO® Cloning |
| CopyCutter™ EPI400™ (Epicentre, USA) | *F- mcrA Δ(mrr-hsdRMS-mcrBC) Φ80dlacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara, leu)7697 galU galK λ- rpsL (StrR) nupG trfA tonA pcnB4 dhfr* | Used for cloning of coding sequences which are toxic to *E.coli* |
| Origami-2 (DE3) (Merck, D) | *Δ(ara-leu)7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL F' [lac+ lacIq pro] (DE3) gor 522::Tn 10 trxB (StrR, TetR)* | Used for protein expression |
| DB3.1™ (invitrogen) | *F-gyr*A462 *end*A1 Δ(sr1-*rec*A) *mcr*B *mrr hsd*S20(rB–, mB–) *sup*E44 *ara*-14 *gal*K2 *lac*Y1 *pro*A2*rps*L20(SmR) *xyl*-5 λ– *leu mtl*1 | Used for amplification of *Donor* and *Destination* vectors |

### 2.1.2 Agrobacterium thumefaciens strains

GV3101::pMP90 (Koncz and Schell, 1986)

The strain GV3101::pMP90 is a rifampicin resistant derivate of *Agrobacterium thumefaciens* C58C1 of which the Ti-plasmid pTiC58 *traC* was removed. Instead the strain was supplemented with the plasmid pMP90. pMP90 is a derivate of pTiC58 *traC nocC* in which the T-region was completely replaced by a gentamycine resistence operon.

### 2.1.3 *Saccharomyces cerevisiae* strains

table 2.2: Saccharomyces cerevisiae strains
for mating-based split-ubiquitin (mbSUS) and yeast two-hybrid (Y2H) assays

| strain (source) | genotype | function |
|---|---|---|
| THY.AP4 (Grefen *et al.*, 2009) | MATa; *ade2−, his3−, leu2−, trp1−, ura3−; lexA::ADE2, lexA::HIS3, lexA::lacZ* | Used for mbSUS |
| THY.AP5 (Grefen *et al.*, 2009) | MATα, *ade2−, his3−, leu2−, trp1−* | Used for mbSUS |
| pJ69-4A (James *et al.*, 1996) | MATa, *trp*1-901, *leu*2-3, 112 *ura*3-52, *his*3-200, *gal4*Δ, *gal*80Δ, LYS2::GAL1-GAL2-*ADE*2, *met*2::GAL7-*lacZ* | Used for Y2H |

### 2.1.4 *Arabidopsis thaliana* lines

#### 2.1.4.1 *Arabidopsis thaliana* lines which have been provided for the Ph.D. thesis

table 2.3: *Arabidopsis thaliana* lines which have been provided for the Ph.D. thesis

| line (NASC) | ecotype | description | source |
|---|---|---|---|
| Nos-0 | Nos-0 | wildtype | Paul Verslues |
| *ahk1-1* | Nos-0 | Kumar *et al.* (2013) | Paul Verslues |
| Ws-2 | Ws-2 | wildtype | Katharina Caesar |
| *ahk1-3* | Ws-2 | Wohlbach *et al.* (2008) | Katharina Caesar |
| *ahk1-4* | Ws-2 | Wohlbach *et al.* (2008) | Katharina Caesar |
| *ahk1-3*/*35S::AHK1-GFP* | Ws-2 | *pH7FWG2-AHK1* (vector #1168) in *ahk1-3*, homozygote | Katharina Caesar |
| *bri1-5* | Ws-2 | Noguchi *et al.* (1999) | Peter Huppenberger |
| *bak1-1* (N6125) | Ws-2 | Li *et al.* (2002) | NASC |
| Col-0 | Col-0 | wildtype | Paul Verslues |
| *ahk1-5* | Col-0 | Kumar *et al.* (2013) | Paul Verslues |
| *ahk1-6* | Col-0 | Kumar *et al.* (2013) | Paul Verslues |
| AHK1 ox | Col-0 | *pUBQ10::AHK1-GFP* (vector #1708) in Col-0 | Katharina Caesar |
| *bri1-201* (N9532) | Col-0 | Domagalska *et al.* (2007) | Sacco de Vries |
| *bri1-301* | Col-0 | Kang *et al.* (2010) | Sacco de Vries |
| *cngc7* (N679395) | Col-0 | SALK_019117.56.00.x | NASC |
| *bak1-3* (N534523) | Col-0 | Kemmerling *et al.* (2007) | Birgit Kemmerling |
| *bak1-4* | Col-0 | Kemmerling *et al.* (2007) | Birgit Kemmerling |
| *aha1-6* (N67805) | Col-0 | Haruta *et al.* (2010) | Friederike Wanke |
| *aha2-4* (N67807) | Col-0 | Haruta *et al.* (2010) | Friederike Wanke |
| *ahk2 ahk3* | Col-0 | Higuchi *et al.* (2004) | Virtudes Mira- Rodado |
| Col-0/R-GECO1 (1460/1) | Col-0 | unpublished | Karin Schuhmacher |

#### 2.1.4.2 *Arabidopsis thaliana* lines which have been generated during the Ph.D. thesis

table 2.4: *Arabidopsis thaliana* lines which have been generated during the Ph.D. thesis

| line | ecotype | description | source |
|---|---|---|---|
| pHK1::mC | Col-0 | *pB7-AHK1pro-mCherryNLS* (vector #1868) in Col-0 | *A. thumefaciens* transformation |
| Ws-2.RFP-MBD | Ws-2 | *pUBN-RFP-MBD* (vector #3296) in Ws-2, T2, heterozygote | *A. thumefaciens* transformation |
| *ahk1-3*.RFP-MBD | Ws-2 | *pUBN-RFP-MBD* (vector #3296) in *ahk1-3*, T2, heterozygote | *A. thumefaciens* transformation |
| *ahk1-4*.RFP-MBD | Ws-2 | *pUBN-RFP-MBD* (vector #3296) in *ahk1-4*, T2, heterozygote | *A. thumefaciens* transformation |
| Ws-2.ABD2-GFP | Ws-2 | *pUB-GFP-ABD2-GFP* (vector #3298) in Ws-2, T2, heterozygote | *A. thumefaciens* transformation |
| *ahk1-3*.ABD2-GFP | Ws-2 | *pUB-GFP-ABD2-GFP* (vector #3298) in *ahk1-3*, T2, heterozygote | *A. thumefaciens* transformation |
| *ahk1-4*.ABD2-GFP | Ws-2 | *pUB-GFP-ABD2-GFP* (vector #3298) in *ahk1-4*, T2, heterozygote | *A. thumefaciens* transformation |
| *bri1-5 ahk1-3* | Ws-2 | homozygoute | crossing |
| *bak1-1 ahk1-3* | Ws-2 | homozygote | crossing |

### 2.1.5 *Nicotiana benthamiana* lines

*Nicotiana benthamiana L. Samsun NN*

## 2.2 DNA

### 2.2.1 Vectors

#### 2.2.1.1 Vectors which have been provided for the Ph.D. thesis

A complete list of the vectors which have been provided for the Ph.D. thesis is included in appendix (A1). Maps of the vectors which show the important functional features are attached in appendix (A3).

#### 2.2.1.2 Vectors which have been generated during the Ph.D. thesis

A list of the vectors which were generated during the Ph.D. thesis is included in the appendix (A2). Maps of the vectors which show the important functional features are attached in appendix (A3).

### 2.2.2 Oligonucleotides

Oligonucleotides were ordered from biomers.net.

The appendix comprises the list of oligonucleotides for genotyping of *Arabidopsis thaliana* T-DNA insertion lines (A4), oligonucleotides and restriction enzymes for genotyping of *Arabidopsis thaliana* EMS mutants (A5), oligonucleotides for the detection of T-DNAs in stably transformed *Arabidopsis thaliana* lines (A6), oligonucleotides for cloning (A7), oligonucleotides for site-directed mutagenesis (A8) and oligonucleotides for sequencing by GATC-Biotech (A9).

## 2.3 General chemicals and solutions

### 2.3.1 Chemicals

Unless otherwise noted, all used chemicals were ordered analytically pure from Sigma-Aldrich (Steinheim, D) and Roth (Karlsruhe, D).

### 2.3.2 Antibiotics

table 2.5: Antibiotics

| antibiotic | selection *E.coli* | selection *A. thumefaciens* | selection *A. thaliana* | solvent | company |
|---|---|---|---|---|---|
| Ampicillin | 100µg/mL | - | - | 70% EtOH | Roth® |
| Kanamycin | 50µg/mL | 50µg/mL | 50µg/mL | $H_2O$ | Roth® |
| Spectinomycin | 50µg/mL | 100µg/mL | - | $H_2O$ | AppliChem |
| Hygromycin | - | - | 25µg/mL | $H_2O$ | Sigma-Aldrich |
| Rifampicin | - | 100µg/mL | - | DMSO | Sigma-Aldrich |
| Gentamycin | 10µg/mL | 40µg/mL | - | $H_2O$ | Duchefa |

### 2.3.3 Hormones and inhibitors

table 2.6: Hormones and inhibitors

| hormone | solvent | company |
|---|---|---|
| 1-aminocyclopropane-1-carboxylic acid | $H_2O$ | Sigma-Aldrich |
| silver nitrate | $H_2O$ | Biotech |
| methyl-jasmonate | ethanol | Sigma-Aldrich |
| salicylic acid | ethanol | Sigma-Aldrich |
| indole-3-acetic acid | ethanol | Serva |
| abscissic acid | ethanol | Sigma-Aldrich |
| brassinolide | ethanol | Sigma-Aldrich |
| propiconazole | ethanol | Sigma-Aldrich |
| β-estradiol | ethanol | Sigma-Aldrich |
| kinetin | $H_2O$ | Sigma-Aldrich |
| 1-N-naphthylphthalamic acid | DMSO | Sigma-Aldrich |

### 2.3.4 Elicitors (PAMPs)

The pathogen-associated molecular pattern flg22 was kindly provided by Markus Albrecht (ZMBP, Biochemistry).

### 2.3.5 Enzymes and commercial kits

table 2.7: Enzymes and commercial kits

| enzyme or commercial kit | company |
|---|---|
| *Taq* DNA Polymerase | New England Biolabs |
| Phusion® High Fidelity DNA Polymerase | Thermo Scientific |
| T4-DNA-Polymerase | Thermo Scientific |
| pENTR™/D-TOPO® Cloning Kit | Thermo Scientific |

| enzyme or commercial kit | company |
|---|---|
| Gateway® LR Clonase enzyme mix | Thermo Scientific |
| Gateway® BP Clonase enzyme mix | Thermo Scientific |
| restriction endonucleases | Thermo Scientific and New England Biolabs |
| Shrimp Alkaline Phosphatase, SAP | Thermo Scientific |
| T4 Polynucleotide Kinase, PNK | Thermo Scientific |
| T4 DNA Ligase | Thermo Scientific |
| RevertAid™ H Minus Reverse Transcriptase | Thermo Scientific |
| PureLink™ Quick Gel Extraction Kit | Invitrogen |
| Gel Extraction Kit | genaxxon |
| EURx GeneMATRIX Universal RNA Purification Kit | roboklon |
| NucleoBond Xtra Midi (50) | Macherey-Nagel |
| Maxima® SYBR Green qPCR Master Mix (2X) | Thermo Scientific |

### 2.3.6 Antibodies

table 2.8: Antibodies

| antibody | clonality | source organism | dilution for use | company |
|---|---|---|---|---|
| α-c-myc | monoclonal | mouse | 1:1000 | Roche |
| α-HA | monoclonal | rat | 1:1000 | Roche |
| α-VP16 | polyclonal | rabbit | 1:500 | GeneTex |
| α-mouse-AP | polyclonal | goat | 1:3000 | BioRad |
| α-rat-AP | polyclonal | goat | 1:3000 | Sigma |
| α-rabbit-AP | polyclonal | goat | 1:3000 | Sigma |
| α-His-AP | monoclonal | mouse | 1:3000 | antibodies-online |

### 2.3.7 Size standards

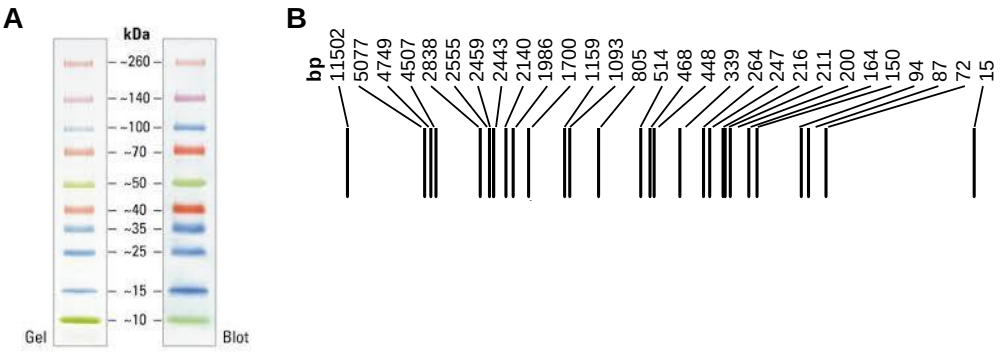



figure 2.1: Protein and DNA size standards
(A) SpectraTM Multicolor Broad Range Protein Ladder (Thermo Scientific). Bands mark proteins of the sizes 260kDa, 140kDa, 100kDa, 70kDa, 50kDa, 40kDa, 35kDa, 25kDa, 15kDa and 10kDa. (B) Self-made λ-PstI DNA size marker. Bands mark DNA-fragments of the sizes 11502bp, 5077bp, 4749bp, 4507bp, 2838bp, 2555bp, 2459bp, 2443bp, 2140bp, 1986bp, 1700bp, 1159bp, 1093bp, 805bp, 514bp, 468bp, 448bp, 339bp, 264bp, 247bp, 216bp, 211bp, 200bp, 164bp, 150bp, 94bp, 87bp, 72bp and 15bp from the left to the right.

table 2.9: Size standards

| description | size standard for | company |
|---|---|---|
| *λ*-PstI marker | DNA | self-made |
| Spectra™ Multicolor Broad Range Protein Ladder | protein | Thermo Scientific |

## 2.4 General solutions

| | | | |
|---|---|---|---|
| 10X TE-buffer pH 8.0 | 100mM | Tris/HCl pH 8.0 | |
| | 10mM | EDTA | |
| TE-buffer pH 8.0 | 10% (v/v) | 10X TE-buffer pH 8.0 | |
| 0.5M NaOH | | | |
| 1.5M Tris/HCl pH 8.8 | | | |
| 1M Tris/HCl pH 8.0 | | | |
| 0.5 Tris/HCl pH 6.8 | | | |
| 20% SDS | | | |

## 2.5 Special buffers and solutions for work with bacteria

### 2.5.1 Growth media

| | | | |
|---|---|---|---|
| Luria-Bertani broth (LB) | 1% (w/v) | Bacto-Peptone | |
| | 0.5% (w/v) | Yeast extract | |
| | 1% (w/v) | NaCl | |
| for plates add | 1.5% (w/v) | Agar No.1 (Oxoid) | before autoclaving |
| Yeast Extract Broth (YEB) | 0.5% (w/v) | Beef Extract | |
| | 0.1% (w/v) | Yeast Extract | |
| | 0.5% (w/v) | Peptone | |
| | 0.5% (w/v) | Sucrose | |
| | 2mM | $MgSO_4$ | |
| for plates add | 1.5% (w/v) | Agar No.1 (Oxoid) | before autoclaving |

For the production of selection media respective antibiotics were added when the autoclaved media had ~55°C at most.

### 2.5.2 Media and buffers to obtain chemically competent cells

| | | |
|---|---|---|
| Super Optimal Broth (SOB) | 2% (w/v) | Bacto Tryptone |
| | 0.5% (w/v) | Yeast Extract |
| | 10mM | NaCl |
| | 2.5mM | KCl |
| | adjust pH with NaOH to pH 7.0 | |
| after autoclaving add | 10mM | $MgCl_2$ (1M stock, sterile filtrated) |
| | 10mM | $MgSO_4$ (1M stock, sterile filtrated) |

| | | | |
|---|---|---|---|
| RF1 | 100mM | RbCl | |
| | 50mM | $MnCl_2$ | |
| | 30mM | Potassium acetate | |
| | 10mM | $CaCl_2$ | |
| | 15% (v/v) | glycerol | |
| | adjust pH with acetic acid to pH 5.8 | | |
| | sterilize by filtration | | |
| RF2 | 10mM | MOPS | |
| | 10mM | RbCl | |
| | 75mM | $CaCl_2$ | |
| | 15% (v/v) | glycerol | |
| | adjust pH with KOH and HCl to pH 6.1 – 6.4 | | |
| | sterilize by filtration | | |

## 2.6 Special buffers and solutions for work with yeast

### 2.6.1 Growth media

| | | | | |
|---|---|---|---|---|
| YPAD | | 2% (w/v) | Peptone | |
| | | 2% (w/v) | Glucose | |
| | | 1% (w/v) | Yeast Extract | |
| | | 0.001% (w/v) | Adenine-Hydrochloride | |
| | for plates add | 2% (w/v) | Agar No.1 (Oxoid) | before autoclaving |
| CSM-$Leu^-$-$Trp^-$ | | 2% (w/v) | Glucose | |
| | | 0.5% (w/v) | $(NH)_4SO_4$ | |
| | | 0.17% (w/v) | Yeast Nitrogen Base (Becton, Dickinson) | |
| | | 0.064% (w/v) | CSM-$Leu^-$-$Trp^-$ (Th.Geyer, D) | |
| | for plates add | 2% (w/v) | Agar No.1 (Oxoid) | before autoclaving |
| CSM-$Leu^-$-$Trp^-$-$Ade^-$ | | 2% (w/v) | Glucose | |
| | | 0.5% (w/v) | $(NH)_4SO_4$ | |
| | | 0.17% (w/v) | Yeast Nitrogen Base (Becton, Dickinson) | |
| | | 0.064% (w/v) | CSM-$Leu^-$-$Trp^-$-$Ade^-$ (Th.Geyer, D) | |
| | | 2% (w/v) | Agar No.1 (Oxoid) | |
| CSM minimal medium | | 2% (w/v) | Glucose | |
| | | 0.5% (w/v) | $(NH)_4SO_4$ | |
| | | 0.17% (w/v) | Yeast Nitrogen Base (Becton, Dickinson) | |
| | | 0.064% (w/v) | CSM-$Ade^-$-$His^-$-$Trp^-$-$Leu^-$-$Ura^-$-$Met^-$ (Th.Geyer, D) | |
| | for plates add | 2% (w/v) | Agar No.1 (Oxoid) | before autoclaving |
| 0.2% Ade | | 0.2g | adenine sulfate | |
| | | 100mL | MilliQ water | |
| | sterilize by filtration, store at 4°C | | | |
| 0.2% Ura | | 0.2g | uracile | |
| | | 100mL | MilliQ water | |
| | sterilize by filtration, store at 4°C | | | |

| | | | |
|---|---|---|---|
| 1% Leu | 1.0g | L-leucine | |
| | 100mL | MilliQ water | |
| | sterilize by filtration, store at 4°C | | |
| 1% Trp | 1.0g | L-tryptophane | |
| | 100mL | MilliQ water | |
| | sterilize by filtration, store at 4°C | | |
| 1% His | 1.0g | L-histidine | |
| | 100mL | MilliQ water | |
| | sterilize by filtration, store at 4°C | | |
| 1.5% Met | 1.5g | L-methionine | |
| | 100mL | MilliQ water | |
| | sterilize by filtration, store at 4°C | | |
| CSM-Ade$^+$-His$^+$-Trp$^+$-Ura$^+$ | 100% (v/v) | CSM minimal medium | |
| for plates add | 2% (w/v) | Agar No.1 (Oxoid) | before autoclaving |
| | autoclave | | |
| | 1% (v/v) | 0.2% Ade | |
| | 0.2% (v/v) | 1% His | |
| | 0.2% (v/v) | 1% Trp | |
| | 1% (v/v) | 0.2% Ura | |
| CSM-Ade$^+$-His$^+$-Leu$^+$ | 100% (v/v) | CSM minimal medium | |
| for plates add | 2% (w/v) | Agar No.1 (Oxoid) | before autoclaving |
| | autoclave | | |
| | 1% (v/v) | 0.2% Ada | |
| | 0.2% (v/v) | 1% His | |
| | 1% (v/v) | 1% Leu | |
| CSM-Ade$^+$-His$^+$ | 100% (v/v) | CSM minimal medium | |
| for plates add | 2% (w/v) | Agar No.1 (Oxoid) | before autoclaving |
| | autoclave | | |
| | 1% (v/v) | 0.2% Ade | |
| | 0.2% (v/v) | 1% His | |
| CSM-Met$^+$ | 100% (v/v) | CSM minimal medium | |
| for plates add | 2% (w/v) | Agar No.1 (Oxoid) | before autoclaving |
| | autoclave | | |
| | 0.05% (v/v) | 1.5% Met | |

### 2.6.2 Buffers for the transformation of *S. cerevisiae*

| | | |
|---|---|---|
| salmon sperm DNA | 400mg | Salmon Sperm DNA (Sigma-Aldrich) |
| | *ad* 50mL | sterile TE/LiAc buffer pH7.5 |
| | Incubate at 4°C for 24-48h while rotating. | |
| | Store Aliquots at -20°C. | |

| | | |
|---|---|---|
| LiAc stock solution | 1M | LiAc |
| | adjust pH to pH 7.5 with acetic acid | |
| | autoclave | |
| PEG stock solution | 50% (w/v) | PEG 4000 |
| | autoclave | |
| 10X TE buffer | 100mM | Tris/HCl pH 7.5 |
| | 10mM | EDTA |
| | autoclave | |
| TE/LiAc buffer | 10% (v/v) | 10X TE buffer |
| | 10% (v/v) | 1M LiAc stock solution |
| PEG/LiAc buffer | 10% (v/v) | 1M LiAc stock solution |
| | 10% (v/v) | 10X TE buffer |
| | 80% (v/v) | PEG stock solution |

## 2.7 Special buffers and solutions for work with plants

### 2.7.1 Growth substrates and media

| | | |
|---|---|---|
| ½ MS | 2.15g/L | Murashige and Skoog basal salt mixture (Sigma-Aldrich) |
| | adjust pH with KOH to pH 5.7 | |
| | autoclave | |
| ½ MS-agar | 2.15g/L | Murashige and Skoog basal salt mixture (Sigma-Aldrich) |
| | adjust pH with KOH to pH 5.7 | |
| | 1% (w/v) | Phytoagar (Duchefa) |
| | autoclave | |

For ½ MS-agar supplemented with hormones, PAMPs and inhibitors the substances were added to autoclaved ½ MS-agar of ~55°C.

| | | | |
|---|---|---|---|
| 5X MS-stock | 21,5g/L | Murashige and Skoog basal salt mixture (Sigma-Aldrich) | |
| Osmotic stress media | 10% (v/v) | 5X MS-stock | |
| | 0.039% (w/v) | MES | |
| | add respective mass of mannitol/sorbitol/$CaCl_2$ | | |
| | adjust pH with KOH to pH 5.7 | | |
| for plates add | 1% (w/v) | Phytoagar (Duchefa) | before autoclaving |

| | | |
|---|---|---|
| JPL-medium | 10% (v/v) | 10X Medium Stock Solution |
| | 0,3% (v/v) | 1M MES pH 5.8 |
| | 0.5% (w/v) | Sucrose |
| | 2mM | $KNO_3$ |
| | 1mM | $NH_4NO_3$ |
| | 1mM | glutamine |
| | 2mM | $K_2SO_4$ |
| | 4mM | $CaCl_2$ |
| | 1mM | $MgSO_4$ |
| 10X Medium Stock Solution | 0.3% (v/v) | Microelement Stock Solution |
| | 0.5% (v/v) | Solution E |
| | 375mM | $KH_2PO_4$ |
| | 0.1mM | Phosphate buffer |
| Microelement Stock Solution | 100mM | $H_3BO_3$ |
| | 100mM | $MnSO_4$ |
| | 36mM | $ZnSO_4$ |
| | 5mM | KI |
| | 1mM | $Na_2MoO_4$ |
| | 0.1mM | $CoCl_2$ |
| | 0.1mM | $CuSO_4$ |
| Solution E | 10mM | $FeSO_4$ |
| | 10mM | $Na_2EDTA$ |
| Phosphate buffer | 39mL | 200mM $NaH_2PO_4$ |
| | 61mL | 200mM $Na_2HPO_4$ |

For plant growth assays on plates, plates in the size of 12cm x 12cm have been used and filled with ~50ml media. Any additives to the media were given when the media was not warmer than 55°C.
For cultivation of *Arabidopsis thaliana* on soil, ten parts T-soil, ten parts P-soil and one part sand were mixed. For the cultivation of *Nicotiana benthamiana* the mixture of T-soil to P-soil to sand was 1:1:1.
T-soil had a pH5.5-6.5 and contained 3.0g/L salt, 250-300mg/L N, 250-450mg/L $P_2O_5$ and 300-500mg/L $K_2O$. P-soil had a pH5.5-6.5 and contained 1.5g/L salt, 100-250mg/L N, 100-250mg/L $P_2O_5$ and 100-250mg/L $K_2O$.

### 2.7.2 Seed surface sterilization

| | | |
|---|---|---|
| sodium hypochlorite solution | 50% (v/v) | Sodium hypochlorite |
| | 0.01% (v/v) | Triton-X-100 |
| ethanol solution | 70% (v/v) | ethanol |
| | 0.01% (v/v) | Triton-X-100 |

### 2.7.3 Stable transformation of *Arabidopsis thaliana* plants

| | | |
|---|---|---|
| transformation solution | 5% | Sucrose |
| | 0.01% | Silwet L-77 |
| | 200µM | Acetosyringon |
| | 10mM | $MgSO_4$ |

### 2.7.4 Transient expression of proteins in *Nicotiana benthamiana*

| | | |
|---|---|---|
| transformation solution | 1% (v/v) | 1M MES/KOH pH 5.6 |
| | 0.1% (v/v) | 200mM Acetosyringon |
| | 0.33% (v/v) | 3M $MgCl_2$ |

### 2.7.5 Induction of protein expression in N. benthamiana with β-estradiol

| | | |
|---|---|---|
| induction solution | 20µM | β-estradiol |
| | 0.1% (v/v) | Tween 20 |

### 2.7.6 Pathogen assay with *Alternaria brassicicola*

| | | |
|---|---|---|
| trypan blue staining solution | 10mL | lactic acid |
| | 10mL | glycerol |
| | 10mL | phenol |
| | 10mL | water |
| | 300mg | trypan blue |
| | 80mL | ethanol |
| chloral hydrate solution | 25g | chloral hydrate |
| | 25mL | water |

### 2.7.7 Destaining of A*rabidopsis thaliana* with acidified methanol

| | | |
|---|---|---|
| acidified methanol | 20% (v/v) | methanol |
| | 4% (v/v) | 37% HCl |
| neutralisation solution | 7% (w/v) | NaOH |
| | 60% (v/v) | ethanol |

### 2.7.8 Protoplast isolation

| | | |
|---|---|---|
| enzyme solution | 10% (v/v) | 0.2M MES pH 5.7 |
| | 10% (v/v) | 0.2M KCl |
| | 50% (v/v) | 0.8M mannitol |
| | 1.5% (w/v) | Cellulase R10 |
| | 0.4% (w/v) | Macerozyme R10 |
| | incubate at 55°C for 10min, then place on ice | |
| add | 10% (v/v) | 0.1M $CaCl_2$ |
| | 0.1% (v/v) | 1mg/mL BSA |
| W5 solution | 1% (v/v) | 0.2M MES pH 5.7 |
| | 2.5% (v/v) | 0.2M KCl |
| | 10% (v/v) | 1.25M $CaCl_2$ |
| | 10% (v/v) | 1.54M NaCl |
| MMG solution | 2% (v/v) | 0.2M MES pH 5.7 |
| | 8% (w/v) | mannitol |
| | 10% (v/v) | 0.15M $MgCl_2$ |

| | | | |
|---|---|---|---|
| 300M solution | | 10mM | MES/KOH pH5.8 |
| | | 10mM | $CaCl_2$ |
| | | 10mM | KCl |
| | | 300mM | mannitol |
| 300ME solution | | 10% (v/v) | 0.2M MES/KOH pH 5.7 |
| | | 10% (v/v) | 0.2M KCl |
| | | 37.5% (v/v) | 0.8M mannitol |
| | | 1.5% (w/v) | Cellulase Onozuka R-10 (Duchefa) |
| | | 0.4% (w/v) | Macerozyme R-10 (Duchefa) |
| | | incubate at 55°C for 10min | |
| | add | 10% (v/v) | 0.1M $CaCl_2$ |

### 2.7.9 Transformation of *Arabidopsis thaliana* mesophyll protoplasts

| | | |
|---|---|---|
| PEG solution | 40% (w/v) | PEG 4000 |
| | 25% (v/v) | 0.8M mannitol |
| | 8% (w/v) | 1.25M $CaCl_2$ |
| luciferin | 20mM | luciferin |
| | solve in water and add KOH until yellow color stays | |

### 2.7.10 Protoplast swelling assay

| | | |
|---|---|---|
| 150M solution | 10mM | MES/KOH pH5.8 |
| | 10mM | $CaCl_2$ |
| | 10mM | KCl |
| | 150mM | mannitol |

## 2.8 Special buffers and solutions for work with RNA

### 2.8.1 Reverse transcription

| | | |
|---|---|---|
| dNTP Mix | 10mM | dATP |
| | 10mM | dTTP |
| | 10mM | dGTP |
| | 10mM | dCTP |

## 2.9 Special buffers and solutions for work with DNA

### 2.9.1 Preparation of plasmid DNA from *Escherichia coli*

| | | |
|---|---|---|
| Mini I | 50mM | Tris/HCl pH8.0 |
| | 10mM | EDTA |
| | autoclave | |
| | 20mg/mL | RNase A |
| Mini II | 0.2M | NaOH |
| | 1% | SDS |

| | | |
|---|---|---|
| Mini III (pH 5.5) | 29.44% (w/v) | $KCH_3COO$ |
| | 11.4% (v/v) | Acetic acid glacial |

### 2.9.2 Extraction of genomic DNA from *Arabidopsis thaliana*

| | | |
|---|---|---|
| Edward's buffer | 200mM | Tris/HCl pH 7.5 |
| | 250mM | NaCl |
| | 25mM | EDTA |
| | 0.5% (w/v) | SDS |

### 2.9.3 Agarose-gel-electrophoresis

| | | |
|---|---|---|
| 50X TAE-buffer | 2M | Tris |
| | 1M | acetic acid |
| | 0.05M | EDTA |
| 1X TAE-buffer | 2% (v/v) | 50X TAE-buffer (40mM Tris, 20mM acetic acid, 1mM EDTA) |

### 2.9.4 Polymerase chain reaction (PCR)

| | | |
|---|---|---|
| dNTP Mix | 10mM | dATP |
| | 10mM | dTTP |
| | 10mM | dGTP |
| | 10mM | dCTP |

## 2.10 Special buffers and solutions for work with proteins

### 2.10.1 Extraction buffers

| | | |
|---|---|---|
| Lyse and Load buffer | 0.05M | Tris/HCl pH 6.8 |
| | 0.1M | DTT |
| | 8M | Urea |
| | 0.005% | Bromophenol blue |
| | 4% | SDS |
| | 30% | glycerol |
| | stored at -20°C | |

### 2.10.2 SDS-PAGE

| | | |
|---|---|---|
| Bottom buffer | 1M | Tris/HCl pH 8.0 |
| | 0.27% (w/v) | SDS |
| | sterilize by filtration | |
| Upper buffer | 0.25M | Tris/HCl pH 6.8 |
| | 0.2% (w/v) | SDS |
| | sterilize by filtration | |

table 2.10: Incredients for of two SDS-PAGE running and stacking gels

| Running Gel | 30% acrylamide | MilliQ water | Bottom buffer | 10% APS | TEMED |
|---|---|---|---|---|---|
| 15.0% | 6.0mL | 1.4mL | 4.5mL | 100µL | 8µL |
| 12.5% | 5.0mL | 2.4mL | 4.5mL | 100µL | 8µL |
| 10.0% | 4.0mL | 3.4mL | 4.5mL | 100µL | 8µL |
| 7.5% | 3.0mL | 4.4mL | 4.5mL | 100µL | 8µL |

| Stacking Gel | 30% acrylamide | MilliQ water | Upper buffer | 10% APS | TEMED |
|---|---|---|---|---|---|
| 4.5% | 0.6mL | 1.4mL | 2.0mL | 20µL | 4µL |

APS: Ammonium persulfate

TEMED: tetramethylethylendiamine

### 2.10.3 Coomassie staining

| | | |
|---|---|---|
| staining solution | 25% (v/v) | isopropanol |
| | 10% (v/v) | acetic acid |
| | 0.05% (w/v) | Coomassie R-250 |
| destainer solution | 10% (v/v) | acetic acid |

### 2.10.4 Western Blot

| | | |
|---|---|---|
| transfer buffer | 1.43% (w/v) | glycine |
| | 0.39% (w/v) | Tris-base |
| | 20% (v/v) | ethanol |

PVDF-membrane (Millipore)

Whatman®-paper (GE Healthcare Life Sciences)

### 2.10.5 Immunodetection

| | | |
|---|---|---|
| 10X TBS buffer | 0.5M | Tris/HCl pH 7.4 |
| | 1.5M | NaCl |
| 1X TBS buffer | 10% (v/v) | 10X TBS buffer |
| TBS-Tween | 10% (v/v) | 10X TBS buffer |
| | 0.1% (v/v) | Tween20 |
| blocking solution | 10% (v/v) | 10X TBS buffer |
| | 5% (w/v) | milk powder |
| staining buffer A | 100mM | Tris/HCl pH 9.5 |
| | 100mM | NaCl |
| | 5mM | $MgCl_2$ |
| NBT stock solution | 5% (w/v) | nitro blue tetrazolium |
| | 70% (v/v) | Dimethylformamid |
| BCIP stock solution | 5% (w/v) | 5-bromo-4-chloro-3-indolylphosphate-p-tuloidin |
| | 100% (v/v) | Dimethylformamid |
| staining solution | 0.66% (v/v) | NBT stock solution |
| | 0.33% (v/v) | BCIP stock solution |
| | 100% (v/v) | staining buffer A |

### 2.10.6 CD-spectroscopy with AHK1-ED

| | | |
|---|---|---|
| buffer for AHK1-ED | 0.25M | Tris/HCl pH 9.0 |
| | 0.15M | NaCl |
| | 0.1% (v/v) | Triton-X-100 |
| | 0.1% (v/v) | sodium lauroyl sarcosine |
| buffer for AHK1-ED-Leu298/422Ala | 0.25M | Tris/HCl pH 9.0 |
| | 0.15M | NaCl |
| | 0.1% (v/v) | sodium lauroyl sarcosine |

## 2.11 Growth chambers for plants

| | |
|---|---|
| long day chamber | 16h light / 8h dark<br>light tubes: 33% Osram L18W/77 Fluora,<br>66% Osram L18/840 Lumilux Cool White<br>20°C (day) / 18°C (night)<br>50% humidity |
| short day chamber | 8h light / 16h dark<br>light tubes: 50% Osram L18W/77 Fluora,<br>50% Osram L18/840 Lumilux Cool White<br>21°C (day) / 20°C (night)<br>50% humidity |
| constant light chamber | 24h light (89µmol $m^{-2}$ $s^{-1}$)<br>20°C |
| greenhouse (*A. thaliana*) | 16h light / 8h dark<br>18°C (day) / 15°C (night)<br>55-60% humidity |
| greenhouse (*N. benthamiana*) | 14h light / 10h dark<br>23°C (day) / 20°C (night)<br>60% humidity |

## 2.12 Machines

Thermomixer 5436, Eppendorf

Vortex-Genie™, Bender & Hobein AG

Sherwood flame photometer Model 410

Roth Micro Centrifuge

Eppendorf Centrifuges 5417R, 5417C, 5810R

Beckmann J2-21M induction drive centrifuge

PCR-Thermocycler PeqStar96 Universal gradient, Peqlab

microscopes: TCS SP2, Leica Microsystems GmbH, TCS SP8, Leica Microsystems GmbH

clean benches: Microflow Biological Safety cabinet, ASTEC

incubators: HettCube 600 R, Hettrich; Inova 44, Eppendorf

Agarose gel-electrophoresis chambers: Peqlab Perfect Blue™ Gelsystem

Labnet Power Station 300 Plus

Scanner: Expression 1600, Epson

NanoDrop photometer ND-1000, NanoDrop products

SDS-PAGE chambers: Mini-PROTEAN® Tetra Vertical Electrophoresis Cell, BioRad

PowerPac™ High-Current Power Supply, BioRad

Mini Trans-Blot Electrophoretic Transfer Cell, BioRad

Silamat® S6, Ivoclar Vivadent

## 2.13 Software

PicsArt

ImageJ (Wayne Rasband, National Institutes of Health)

Gimp (The Gimp Team)

ApE - A plasmid editor (by M. Wayne Davis)

Microsoft Office 2007 + 2010 (Microsoft Corporation)

Adobe Reader IX (Adobe Systems Software Ireland Limited)

Leica Application Suite X (Leica Microsystems GmbH)

Leica Application Suite AF Lite (Leica Microsystems GmbH)

## 2.14 Online resources

| | |
|---|---|
| MUSCLE sequence alignment | http://www.ebi.ac.uk/Tools/msa/muscle/ |
| Pub Med and BLAST | https://www.ncbi.nlm.nih.gov |
| ExPasy translate tool | http://web.expasy.org/translate/ |
| prediction of protein domains | www.elm.eu.org |
| | http://smart.embl-heidelberg.de |
| Arabidopsis eFP browser | http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi |
| information about *Arabidopsis* lines | http://arabidopsis.info/BrowsePage |
| | https://www.arabidopsis.org |

## 2.15 External devices

GATC-Biotech (D)

GenScript (USA)

GeneCust (L)

# 3 METHODS

## 3.1 Molecular-biological methods

### 3.1.1 Production of competent cells

#### 3.1.1.1 Production of chemically competent *Escherichia coli* cells

Cells from a glycerol-stock were distributed on a selection-free LB-plate using 2.85-3.45mm glass beads and grown over night at 37°C. A 5mL LB-preculture was inoculated with one single colony and grown over night at 28°C while shaking. Two 200mL SOB-main-cultures were inoculated in 2L flasks with 0.1mL of the LB-preculture and grown up to an $OD_{600}$ of 0.45 to 0.55 at 25°C while shaking. Before the cells were pelletized by centrifugation at 4°C with 2500g for 10min, the culture was incubated on ice for 15min. The pellet was resuspended in 4°C cold 5mL RF1 and incubated for one hour on ice. The cells were again pelletized by centrifugation at 4°C with 2500g for 10min. The pellet was resuspended in 4°C cold 4mL RF2 and incubated on ice for 15min. Aliquots of 50µl were immediately frozen in liquid nitrogen and stored at -80°C. The analysis of resistance and competence was executed on the day of production and two weeks later. For the analysis of resistance it was checked that the cells do not grow on Ampicillin-, Kanamycin-, Spectinomycin- and Gentamycin-selection.

#### 3.1.1.2 Production of electrically competent *Escherichia coli* cells

Cells from a glycerol-stock were distributed on a selection-free LB-plate using 2.85-3.45mm glass beads and grown over night at 37°C. A 5mL LB-preculture was inoculated with one single colony and grown over night at 37°C while shaking. 100mL main culture were inoculated with the over-night preculture to an $OD_{600}$=0.01. When the $OD_{600}$ reached 0.5 cells were pelletized by centrifugation at 4°C with 4000rpm for 10min. The cells were then washed twice with pre-cooled 90mL sterile MilliQ water and once with pre-cooled 90mL autoclaved 10% glycerol. Subsequenly the cells were resuspended in 1mL autoclaved 10% glycerol. Aliquots of 50µL were directly frozen in liquid nitrogen and stored at -80°C.

#### 3.1.1.3 Production of chemically competent *Agrobacterium thumefaciens* cells

A dilution streaking was executed on YEB-media with Rifampicin (Rif) and Gentamycin (Gent) as selection using a glycerol stock of the *Agrobacterium thumefaciens* strain GV3101. The plates were incubated for two to three days at 28°C. The selection with Rif and Gent was applied in all following cultures. A 5mL YEB-preculture was inoculated with one single colony and incubated over night at 28°C while shaking. For an intermediate culture 22.5mL YEB-medium were inoculated with 2.5mL of the preculture and grown over night at 28°C while shaking. The 25mL of the intermediate culture were then used to inoculate the main culture of 250mL YEB-medium. The main culture was cultured up to an $OD_{600}$ of 0.5 – 0.8. Before the cells were pelletized by centrifugation at 4°C with 4000g for 5min, the

culture was incubated on ice for 15min. The cell pellet was resuspended in 50mL of pre-cooled 150mM $CaCl_2$-solution. The cells were again pelletized by centrifugation at 4°C with 4000g for 5min. The cell pellet was then resuspended in 10mL of pre-cooled 20mM $CaCl_2$-solution. Aliquots of 100µL were immediately frozen in liquid nitrogen and stored at -80°C. For the analysis of resistance it was checked that the cells do not grow on Kanamycin- and Spectinomycin-selection.

#### 3.1.1.4 Production of chemically competent *Saccharomyces cerevisiae*

An over-night preculture of 4-5mL YPAD was inoculated with 1 colony of the respective *Saccharomyces cerevisiae* strain and incubated over night at 28°C shaking. The main culture of 4mL YPAD was inoculated with 0.4mL of the preculture and shaken at 28°C for four to six hours until an $OD_{600}$ of 0.8 – 1.0 was reached. The cells were pelletized by centrifugation at room temperature with 1700g for 1min and resuspended in 500µL TE/LiAc buffer. The centrifugation and resuspension was repeated two to three times. Then the cells were resuspended in 300µL TE/LiAc buffer and incubated on ice for 10min to 3h. The competent cells were always used freshly for transformation.

#### 3.1.1.5 Analysis of competence of competent cells

For the test of the competence of *E. coli* cells a transformation of 50µL competent cells was executed with 1µL of 10pg/µL pUC19. Instead of 1mL LB for the one hour recovering at 37°C just 300µL LB were used. After the recovering 20µL, 50µL and 80µL of the transformed cells were distributed on LB medium with Ampicillin selection using 2.85-3.45mm glass beads and grown over night at 37°C. The grown colonies were counted for the calculation of competence. The transformation efficiency TE is defined as TE = Colonies / µg DNA / dilution and therefore as the number of colony forming units per 1µg of plasmid.

### 3.1.2 Transformation of competent cells

#### 3.1.2.1 Transformation of chemically competent *Escherichia coli*

The aliquot of chemically competent *E. coli* cells (NEB®5α, One Shot® TOP10, Origami-2 (DE3)) was slowly thawed on ice. 0.1-1µg of vector DNA was added to the thawed cells and the mixture was incubated on ice for 5-30min. Then a heat shock of 42°C for 30-60s was executed and afterwards the cells were incubated for another 2min on ice. 1mL LB-medium without any selection was added and the cells were incubated at 37°C for 1h while shaking. The cells were pelletized by centrifugation at 13000rpm for 30s, plated on LB-plates with the respective selection and grown over night at 37°C.

#### 3.1.2.2 Transformation of electrically competent *Escherichia coli*

The aliquot of electrically competent *E. coli* cells (CopyCutter™ EPI400™) was slowly thawn on ice. 0.1-11µg of DNA was added to the thawn cells and the mixture was incubated on ice for 5-30min. The DNA had to be in water or in very low salt buffer. To get rid of salts for example after ligations the DNA solution was dialyzed using dialysis membranes according to the manual. After the incubation on ice the mixture was filled into the electroporation cuvettes and the electroporation was executed using 1.8kV. The mixture was then again placed on ice for 2min. 1mL LB-medium without any selection was added and the cells were incubated at 37°C for 1h while shaking. The cells were pelletized by

centrifugation at 13000rpm for 30s, plated on LB-plates with the respective selection and grown over night at 37°C.

#### 3.1.2.3 Transformation of chemically competent *Agrobacterium thumefaciens*

The aliquot of chemically competent *A. thumefaciens* was slowly thawed on ice. 1-5µg of a binary vector were added to the thawed cells and the mixture was incubated for 5min on ice, for additional 5min in liquid nitrogen and for 5min at 37°C. Then 1mL YEB-medium without any selection was added. For recovery the cells were then shaken at 28°C for 2-4h. The cells were pelletized with 3000rpm in 2min, subsequently plated on YEB-plates with Rifampicin-, Gentamycin- and the vector-specific selection and then cultivated at 28°C for 2-3d.

#### 3.1.2.4 Verification of the expression construct in *Agrobacterium thumefaciens*

To verify the correctness of the nucleotide sequence of the respective expression construct transformed in A*grobacterium thumefaciens*, a "plasmid rescue" was executed. Therefore the plasmids were extracted by an Alkaline Lysis and transformed into *Escherichia coli* Neb®5α cells. 70µL of this transformation reaction were used to inoculate 4mL LB-medium with appropriate antibiotic selection and were grown over night at 37°C shaking. Subsequently the plasmids were extracted by Alkaline Lysis and analyzed using restriction endonucleases, agarose gel electrophoresis and sequencing.

#### 3.1.2.5 Transformation of chemically competent *Saccharomyces cerevisiae*

For the transformation of *Saccharomyces cerevisiae*, 16.5µL competent cells were mixed with 3.5µL salmon sperm DNA which was heated to 95°C for 3min and then cooled on ice for 1-2min, 2µL of vector DNA of ~0.5-1µg/µL and at last with 100µL PEG/LiAc buffer. The mixture was incubated for 30-60min at room temperature shaking with 500rpm. Subsequent to 20min heat shock at 42°C the cells were recovered for 20-30min at 30°C. The cells were pelletized by centrifugation with 1700g for 1min, resuspended in 50µL sterile MilliQ water, plated on auxotrophy selection plates and grown for 2-5d at 28°C (Gietz and Schiestl, 1995).

For yeast-two-hybrid the bait (*pGBKT7*) and prey vector (*pGADT7*) were co-transformed into the *S. cerevisiae* strain pJ69-4A using 1µL vector DNA of each. The transformed cells were plated on CSM-Leu$^-$-Trp$^-$ as the *pGBKT7*-vector complements the Trp auxotrophy and the *pGADT7*-vector the Leu auxtotrophy of pJ69-4A (Fields ad Song, 1989). For the mating-based split-ubiquitin system bait (*pMetYC*) and prey (*pXNubA22*) vectors were transformed solitary into the two different haploid *S. cerevisiae* strains THY.AP4 and THY.AP5. The transformed THY.AP4 were plated on CSM-Ade$^+$-His$^+$-Trp$^+$-Ura$^+$- and THY.AP5 on CSM-Ade$^+$-His$^+$-Leu$^+$ auxotrophy media (Grefen *et al.*, 2009).

### 3.1.3 Storage of bacterial cells

For long-term storage of *E. coli* and *A. thumefaciens* glycerol- and DMSO-stocks were generated. For glycerol-stocks 500µL of the respective over-night culture were mixed with 500µL autoclaved 60% glycerol, incubated at room temperature for 5-10min, frozen in liquid nitrogen and stored at -80°C. For DMSO-stocks 930µL of the respective over-night culture were mixed with 70µL DMSO, incubated at room temperature for 5-10min, frozen in liquid nitrogen and stored at -80°C.

### 3.1.4 Extraction of nucleic acids

#### 3.1.4.1 Extraction of plasmid DNA

The Alkaline Lysis was executed according to Sambrook *et al.* 1989. 4mL LB-medium with an appropriate antibiotic were inoculated with a single *E. coli* colony and incubated over night at 37°C shaking. The cells were pelletized by centrifugation at room temperature with 13000rpm for 30s. The cells were resuspended in 400µL of Mini I-solution. The lysis was executed with the addition of 400µL of Mini II-solution and incubation at room temperature for 4min at most. The neutralization was obtained with the addition of 400µL Mini III-solution and incubation on ice for 5min. Cell fragments and the drop out were removed by centrifugation at 4°C with 13000rpm for 15min. The addition of an equal volume of 2-propanol to the supernatant and a subsequent incubation at -20°C for 20min effects the precipitation of vector-DNA. The vector-DNA was pelletized by centrifugation at 4°C with 13000rpm for 30min, washed with 70% ethanol and dissolved in 54µL 10mM Tris/HCl pH8.0. For the inactivation of DNases the samples were heated up to 65°C for 10min.
Midi Preps for plasmid DNA in higher concentrations and of higher purity were executed using the NucleoBond Xtra Midi (50) Kit (Macherey-Nagel) according to the manual.

#### 3.1.4.2 Extraction of RNA from *Arabidopsis thaliana*

The extraction of RNA from A*rabidopsis thaliana* was executed using the EURx GeneMATRIX Universal RNA Purification Kit (roboklon) according to the manual.

#### 3.1.4.3 Extraction of genomic DNA from *Arabidopsis thaliana*

100mg of plant material which is frozen in liquid nitrogen was grinded with the addition of glass beads with 1.25-1.65mm size and the use of the Silamat® S6 with 4500rpm for 5-10s. 300µL of Edward's buffer were added and the samples incubated at 65°C for 10min. Cell fragments were removed by centrifugation with 13000rpm for 10min. The DNA was precipitated by the addition of an equal volume of 2-propanol to the supernatant. The genomic DNA was pelletized by centrifugation with 13000rpm for 5-30min, washed with 70% ethanol and dissolved in 100µL 10mM Tris/HCl pH8.0. For the inactivation of DNases the samples were heated up to 65°C for 10min. The genomic DNA was stored at -20°C.

### 3.1.5 Restriction of plasmid DNA

For the analytical restriction and the targeted opening of vector DNA for classical cloning suitable restriction endonucleases of New England Biolabs and Thermo Scientific were used according to the manual. A subsequently executed agarose gel electrophoresis elucidated the size of the DNA fragments.

### 3.1.6 Reverse transcription

For the reverse transcription the protocol of the RevertAid™ H Minus Reverse Transcriptase was followed using total RNA as template RNA and Oligo(dT)$_{18}$ (Thermo Scientific) as primer.

### 3.1.7 Polymerase chain reaction (PCR)

The Polymerase chain reaction (PCR) was used to amplify specific DNA-fragments. Dependent on the purpose of the reaction different DNA polymerases were used. The *Taq* DNA Polymerase of New

England Biolabs was used for analytical PCRs, the Phusion® High Fidelity DNA Polymerase of Thermo Scientific was used for the amplification of DNA-fragments which should be used for cloning and the Maxima® SYBR Green qPCR Master Mix (2X) of Thermo Scientific was used for quantitative real time PCR. The PCRs with the different DNA polymerases were executed according to the respective manual.

### 3.1.8 Genotyping

To confirm homozygosity of exchanges of basepairs and T-DNA insertions, the used *Arabidopsis thaliana* lines were genotyped. To genotype plant lines with T-DNA insertions, PCRs were executed on genomic DNA with *Taq* DNA Polymerase (New England Biolabs) and two pairs of primers. The pair of primers in which both primers were gene-specific, detected putative wildtype alleles. The second pair comprised a gene-specific and a T-DNA specific primer to prove the presence of the T-DNA. When an amplicon could be proven just with the first pair of primers, there was no T-DNA insertion at this site indicating a wildtype allele. The detection of an amplicon for both pairs of primers indicated heterozygosity, the detection of an amplicon just with the second pair of primers indicated homozygosity for the T-DNA insertion. For each genotyping reaction a water-, wildtype- and positive control was added.

To genotype plant lines which were derived from EMS mutagenesis and which have single basepair exchanges PCRs on genomic DNA were executed with *Taq* DNA Polymerase (New England Biolabs) and gene-specific primers. Subsequent to amplicons which were detected in agarose gel electrophoresis, the amplicons were cut with restriction endonucleases which cut specifically either the wildtype or the mutated nucleotide sequence. Dependent on the DNA-fragment size the zygosity of the plant line could be concluded.

To proof the presence of stably transformed constructs in *Arabidopsis thaliana* lines, PCRs were executed on genomic DNA with *Taq* DNA Polymerase (New England Biolabs) and T-DNA specific primers.

### 3.1.9 Site-directed mutagenesis

Site-directed mutagenesis used polymerase chain reaction and primers which are modified at the particular site to specifically exchange base-pairs in the nucleotide sequence of DNA-fragments. Therefore a forward and a complementary reverse primer was designed which contained the desired nucleotide sequence. Two PCR reaction setups of 50µL each were mixed according to the manual of the *Taq* DNA Polymerase (New England Biolabs) but just with the forward- or reverse primer respectively. After 10 cycles of PCR 25µL of each reaction setup were mixed. Subsequent to additional 30 PCR cycles the template vector was erased from the reaction setup by the addition of 1µL of the restriction endonuclease DpnI. DpnI was inactivated at 85°C for 10min. 5µL of the reaction setup were then transformed into *E. coli* cells.

### 3.1.10 Dephosphorylation of DNA-fragments

The dephosphorylation of DNA-fragments avoids the re-attachment of sticky ends and therefore reduces the background in classical cloning. For the dephosphorylation the Shrimp Alkaline Phosphatase (Thermo Scientific) was used according to the manufacturer's manual.

### 3.1.11 Phosphorylation of DNA-fragments

The phosphorylation of DNA-fragments facilitates the directed attachment of phosphorylated DNA-fragments with dephosphorylated DNA-fragments in ligations. For the phosphorylation the T4 Polynucleotide Kinase (Thermo Scientific) was used according to the manufacturer's manual.

### 3.1.12 Classical cloning

After specific cleavage, the extraction of the desired DNA-fragment from an agarose gel and after de- and phosphorylation, DNA-fragments with complementary sticky ends were ligated with the T4 DNA Ligase (Thermo Scientific) according to the manufacturer's manual. The applied ratio of vector backbone to DNA-insert depended on the ratio of their size. For the calculation of the molar amount of DNA the following formular was used:

$$\frac{\mathrm{m(DNA-fragment) in\ \mu g} * 10}{\mathrm{size(DNA-fragment) in\ kb} * 6.6} = \mathrm{n\ in\ nmol}$$

### 3.1.13 *Gateway*™-Cloning

The Gateway™ system of Thermo Scientific/invitrogen enables fast and site-specific recombination of DNA-fragments in vectors. The system is advantageous as one *Entry* clone which contains the gene of interest flanked by *att*L sequences serves as donor of the gene of interest for different *Destination* vectors. *Destination* vectors contain the Gateway™ cassette flanked by *att*R sequences for the *in vitro* recombination with the *Entry* clone (LR-reaction) and are vectors with different features for protein expression. The LR-reaction generates expression clones with the gene of interest flanked by *att*B sites. An *in vitro* recombination between the expression clone and a *Donor* vector with the Gateway™ cassette flanked by *att*P sites (BP-reaction) generates an *Entry* clone and a *Destination* vector.

#### 3.1.13.1 *pENTR™/D-TOPO*® Cloning

To generate an *Entry* clone which contains the gene of interest flanked by *att*L sequences, *pENTR™/D-TOPO*® Cloning was used. Therefore the gene of interest was amplified in a polymerase chain reaction using the Phusion® High Fidelity DNA Polymerase of Thermo Scientific with proof reading function. As the *pENTR™/D-TOPO*® Cloning Kit was used for directional TOPO-cloning the gene-specific forward primer needed to have the sequence 3'-CACC-[gene-specific nucleotide sequence]-5'. For the TOPO-reaction 0.5-2.0µL of the PCR reaction were mixed with 0.5µL Salt Solution and 0.5µL *pENTR™/D-TOPO*® Cloning mix. The total volume of the reaction setup was 3.0µL. The reaction was incubated at room temperature for 5min, then placed on ice and transformed into chemically competent One Shot® TOP10 or, after dialysis, into electrically competent CopyCutter™ EPI400™ *E. coli* cells.

#### 3.1.13.2 BP-Cloning

To generate *Entry* clones from expression clones for site-directed mutagenesis or further generation of expression clones, BP-reactions were executed using the respective expression clone and the *Donor* vector *pDONR207* in the concentration of 150ng/µL. 0.5µL of the expression clone, *pDONR207*, BP-Clonase buffer, TE-buffer pH 8.0 and BP Clonase Enzyme Mix was mixed, incubated for at least 1h at room temperature and transformed into chemically competent NEB®5α *E. coli* cells.

#### 3.1.13.3 LR-Cloning

The reaction setup for the generation of expression clones by an LR recombination reaction was 0.5µL of 150ng/µL *Entry* clone, 150ng/µL *Destination* vector, 5X LR Clonase buffer, TE buffer pH 8.0 and LR Clonase Enzyme Mix. The mixture was incubated for at least 1h at room temperature. Subsequently, 1µL Proteinase K was added and the mix incubated for 20min at 37°C. After 10min heat inactivation at 65°C the LR reaction setup was transformed into chemically competent NEB®5α or into electrically competent CopyCutter™ EPI400™ *E. coli* cells.

### 3.1.14 Expression of proteins in *Escherichia coli*

For the expression of proteins in *Escherichia coli* Origami-2 (DE3) cells 4mL LB medium with appropriate antibiotics were inoculated with a single *Escherichia coli* colony which was transformed with the respective expression vector. After shaking incubation over night at 37°C 4mL LB medium with appropriate antibiotics were inoculated with the over-night culture to $OD_{600}$=0.8 and induced with 0.3mM IPTG. After growth at 16°C for 22h the cells were harvested by centrifugation and the proteins extracted.

### 3.1.15 Denaturing extraction of proteins

Proteins were extracted from *Escherichia coli*, *Saccharomyces cerevisiae* and *Nicotiana benthamiana*. For the extraction of protein from *E. coli* and *S. cerevisiae* the $OD_{600}$ was measured first. Then cells of 2mL of the respective culture were pelletized by centrifugation with 13000rpm for 30s and the supernatant was discarded. Approximately 50µL glass beads in the size of 0.25-0.5mm were added to the pellet. Lyse and Load buffer which was pre-heated to 95°C was added in the volume which would have been necessary to reach an $OD_{600}$ of 20. The samples were vortexed for 1min and shaken at 65°C for 10min. Subsequent to centrifugation with 13000rpm for 30s the supernatant was transferred to a new tube and stored at -20°C.

For the extraction of protein from *N. benthamiana* a leaf part in the size of approximately 1cm² was frozen in liquid nitrogen and shredded with glass beads in the size of 1.25-1.65mm and the Silamat® S6 shaking for 5-10s with 4500rpm. 200µL of Lyse and Load buffer which was pre-heated to 95°C was added. The samples were vortexed for 1min and shaken at 95°C for 10min. Subsequent to centrifugation with 13000rpm for 5min the supernatant was transferred to a new tube and stored at -20°C.

The total protein extraction from *Arabidopsis thaliana* for the analysis of the phosphoproteome as well as the tryptic digestion and phosphopeptide enrichment was carried out as described in Wu and Schulze 2015.

### 3.1.16 Phosphoproteomics (by Waltraud X. Schulze)

The experimental procedures are adopted from Dautel *et al.*, 2015 and extended for *ahk1-3*/Ws-2.

$^{15}$N-labeling and a reciprocal experimental design (Kierszniowska *et al.,* 2009) was used for the kinetin approach with *ahk2 ahk3* and Col-0 as well as for the mannitol approach with *ahk1-3* and Ws-2 (fig.3.1 A). In addition, for *ahk1-3* and Ws-2 an experimental approach without $^{15}$N-labeling was executed (fig.3.1 B). In comparison to the metabolic labeling approach, in which the Col-0 and *ahk2 ahk3* sample or Ws-2 and *ahk1-3* sample respectively could be mixed after harvesting, the samples in the not-

labeled approach had to be further processed individually. These experiments were carried out in at least three biological replicates for each combination of $^{14}N/^{15}N$-labeling as well as for the un-labeled approach. Data were averaged between these replicates.

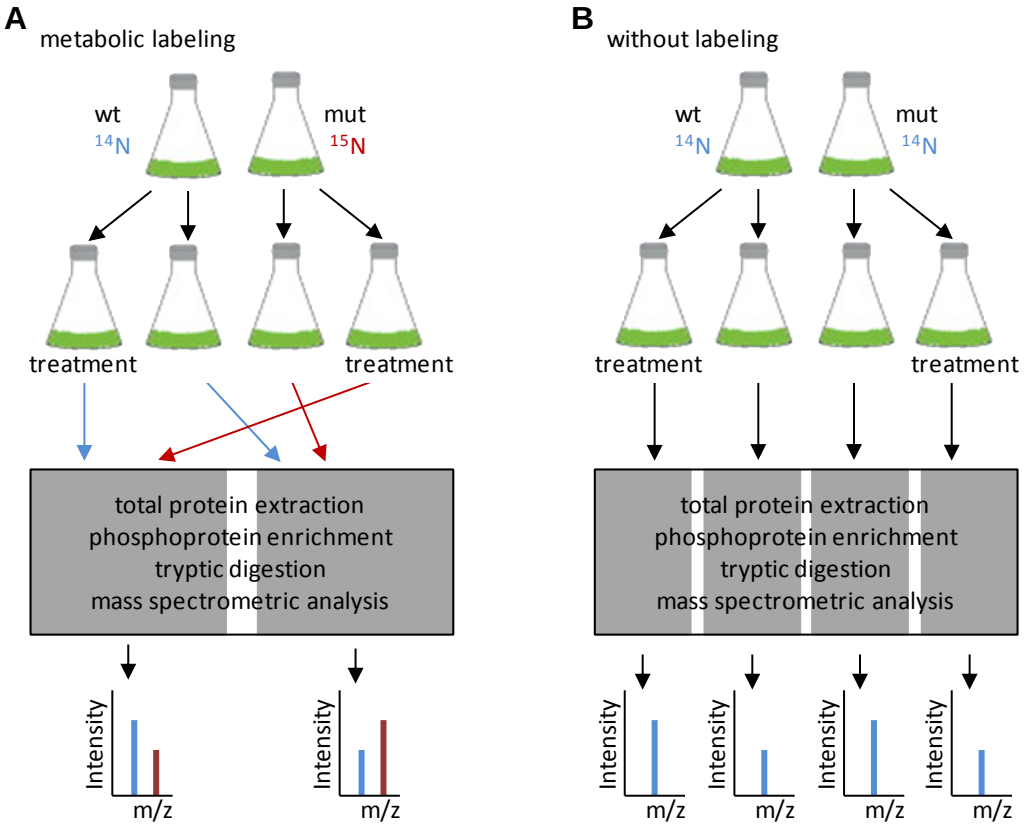



figure 3.1: Experimental setup for the analysis of the phophoproteome detected with and without metabolic labeling.
(A) Experimental setup with metabolic labeling of seedling cultures. Shown is the setup with the unlabeled wildtype (blue) and the metabolic labeled mutant plants (red). The setup was additionally executed *vice versa*. Unlabeled wildtype and labeled mutant plants were treated and 1:1 mixed (A, grey) for total protein extraction, phosphoprotein enrichment, tryptic digestion and mass spectrometric analysis. The mass spectrometric detection of labeled (red) and unlabeled (blue) peptide ions gives an isotope peak separation in light and heavy peaks dependent on the mass-to-charge-ratio (m/z) with quantitative differences in the intensity. A control experiment was carried out without treatment. (B) Experimental setup without labeling of seedling cultures. Treated and untreated cultures of not-labeled (blue) wildtype and mutant plants were used individually (B, grey) for total protein extraction, phosphoprotein enrichment, tryptic digesetion and mass spectrometric analysis. For each culture and treatment individual spectra were measured all together. (A) and (B) were adopted from Arsova *et al.,* 2012 and adjusted.

Protein extraction, tryptic digestion and phosphopeptide enrichment was carried out as described in Wu and Schulze 2015. Tryptic peptide mixtures were analyzed by LC/MS/MS using nanoflow Easy-nLC1000 (Thermo Scientific) as an HPLC-system and a Quadrupole-Orbitrap hybrid mass spectrometer (Q-Exactive Plus, Thermo Scientific) as a mass analyzer. Peptides were eluted from a 75µm x 50cm C18 analytical column (PepMan, Thermo Scientific) on a linear gradient running from 4 to 64% acetonitrile in 120min (240min for label-free samples) and sprayed directly into the LTQ-Orbitrap mass spectrometer. Proteins were identified by MS/MS using information-dependent

acquisition of fragmentation spectra of multiple charged peptides. Up to twelve data-dependent MS/MS spectra were acquired for each full-scan spectrum acquired at 60,000 full-width half-maximum resolution. Overall cycle time was approximately one second (Wu *et al.,* 2014).

For the analysis of the metabolically labeled samples acquired spectra were matched against the *Arabidopsis* proteome (TAIR10, 35386 entries) using Mascot v.2.2. Thereby, carbamidomethylation of cysteine was set as fixed modification; oxidation of methionine as well as phosphorylation of serine, threonine and tyrosine was set as variable modifications. Mass tolerance for the database search was set to 20ppm on full scans and 0.5Da for fragment ions and "$^{15}$N-metabolic labeling" was chosen as quantitation option. Peptides were accepted using a FDR threshold of 0.01. For quantitation, ratios between heavy ($^{15}$N) and light forms of each peptide were calculated using Mascot Distiller. Hits to contaminants (e.g. keratins) and additionally identified reverse hits were excluded from further analysis. Ratios from reciprocal experiments were converted to "mutant vs wildtype" ratios and averaged. Significantly up- or down-regulated proteins were defined by pairwise t-testing with multiple-testing correction (Benjamini *et al.*, 1995) if peptides were identified in both replica experiments. For the peptides identified only in one replica experiment, a two-fold-change cutoff was applied. For the analysis of the not labeled samples protein identification and ion intensity quantitation was carried out by MaxQuant version 1.5.3.8 (Cox *et al.*, 2008). Spectra were matched against the Arabidopsis proteome (TAIR10, 35386 entries) using Andromeda (Cox *et al.*, 2011). Thereby, carbamidomethylation of cysteine was set as a fixed modification. Oxidation of methionine as well as phosphorylation of serine, threonine and tyrosine was set as variable modifications. Mass tolerance for the database search was set to 20 ppm on full scans and 0.5 Da for fragment ions. Multiplicity was set to 1. For label-free quantitation, retention time matching between runs was chosen within a time window of two minutes. Peptide false discovery rate (FDR) and protein FDR were set to 0.01, while site FDR was set to 0.05. Hits to contaminants (e.g. keratins) and reverse hits identified by MaxQuant were excluded from further analysis.

Reported ion intensity values were used for quantitative data analysis. cRacker (Zauber *et al.*, 2012) was used for label-free data analysis of phosphopeptide ion intensities based on the MaxQuant output. All phosphopeptides and proteotypic non-phosphopeptides were used for quantitation. Within each sample, ion intensities of each peptide ions species (each m/z) were normalized against the total ion intensities in that sample (peptide ion intensity/total sum of ion intensities). Subsequently, each peptide ion species (i.e. each m/z value) was scaled against the average normalized intensities of that ion across all treatments. For each peptide, values from three biological replicates then were averaged after normalization and scaling. In case of non-phosphopeptides, protein ion intensity sums were calculated from normalized can scaled ion intensities of all proteotypic peptides.

In this work a $\log_2$ value above 1.0 or beyond -1.0 is set as threshold for differential phosphorylation. For the experiments with metabolic labeling the all phosphopeptides with these $\log_2$ values were included independently from their p-value, as the quantitation of the phosphopeptides was carried out in pairs of *ahk1-3* and wt. For the experiments without labeling just the phosphopeptides with $p<0.05$ were counted.

### 3.1.17 AHA activity assay (by Waltraud X. Schulze)

Proteins were extracted from 14d old seedlings which were grown in liquid culture and treated with 0.3M mannitol and mock for 10min how it was described for the phosphoproteome but without tryptic digestion and phosphopeptide enrichment. The measurement of the change of inorganic phosphate was performed like described in Lanzetta *et. al.* (1979). $Na_3VO_4$ was used as inhibitor for plasma membrane ATPases, EDTA for $Ca^{2+}$ ATPases, $NaN_3$ for mitochondrial ATPases and Bafilomycin A1 for the inhibition of V-ATPases.

## 3.2 Cell-biological methods

### 3.2.1 Cultivation of *Escherichia coli*

For the cultivation of *Escherichia coli* on LB-agar with appropriate antibiotic selection, cells which were dissolved in medium were distributed using sterile 2.85-3.45mm glass beads. The LB-agar plates were incubated over night at 37°C and could be stored for up to three weeks at 4°C.

For the cultivation of *Escherichia coli* in liquid culture 4mL LB-medium with appropriate antibiotic selection were inoculated with a single *E. coli* colony from LB-agar and grown for at least 8h at 37°C shaking. These cultures were directly used for the extraction of plasmid DNA (Alkaline Lysis).

For the production of competent cells as well as for a Midi Prep to extract plasmid DNA the culture was used to inoculate cultures of a bigger volume. These cultures were grown according to the respective protocols.

Liquid cultures of CopyCutter™ EPI400™ cells were used to inoculate 4mL LB-medium with appropriate antibiotic selection and 4µl CopyCutter™ Induction Solution to $OD_{600}$=0.1 to induce plasmid amplification. These cultures were incubated at 37°C for 4h shaking and then used for the extraction of plasmid DNA (Alkaline Lysis).

For the expression of proteins in Origami-2 (DE3) cells, 4mL LB-medium with appropriate antibiotic selection were inoculated with one colony of transformed Origami-2 (DE3) cells and grown over night shaking at 37°C. The preculture was used to inoculate the 5mL LB main culture with appropriate antibiotic selection and 0.3M IPTG to $OD_{600}$=0.8 for the induction of protein expression. The main culture was cultivated for 22-26h shaking at 16°C.

### 3.2.2 Cultivation of *Agrobacterium thumefaciens*

*Agrobacterium thumefaciens* was cultivated on LB as well as on YEB with appropriate antibiotic selection. For the cultivation on plates, cells which were dissolved in medium were distributed using sterile 2.85-3.45mm glass beads whereas a dilution streaking was executed for *A. thumefaciens* of glycerol or DMSO stocks. The plates were incubated for 2-3d at 28°C and could be stored for up to three weeks at 4°C. For the cultivation in liquid culture 4mL medium were inoculated with a single *A. thumefaciens* colony and grown for at least 8h at 28°C shaking.

### 3.2.3 Cultivation of *Saccharomyces cerevisiae*

For the cultivation of Saccharomyces cerevisiae on plates of the appropriate auxotrophy medium dissolved cells were distributed using sterile 2.85-3.45mm glass beads whereas a dilution streaking was executed for S. cerevisiae of glycerol or DMSO stocks. The plates were incubated for 1-5d at

28°C and could be stored for up to three weeks at 4°C. For the cultivation in liquid culture 4mL of the appropriate auxotrophy medium were inoculated with S. cerevisiae, shortly vortexed and incubated at 28°C shaking for at least 8h.

### 3.2.4 Transformation of *Arabidopsis thaliana* plants

For the stable transformation of *Arabidopsis thaliana* plants the “floral dip” method of Clough and Bent, 1998 was used. Therefore a 5mL YEB-preculture with the respective selection was inoculated with one single colony of *A. thumefaciens* carrying the correct vector-DNA which should be transformed into the plant. The preculture was incubated shaking over night at 28°C. The main culture of 200mL YEB-medium with respective selection was inoculated with 4mL preculture and incubated shaking over night at 28°C. The cells of the main culture were pelletized by centrifugation at 4°C with 4000g for 20min and resuspended in 200mL of the transformation solution. For the transformation ten flowering plants in one 8cm pot were put head first into the *Agrobacterium*-transformation-solution for 30s. Then the plants were placed under the tray cover for keeping the humidity. The tray cover was removed the next day. The floral dipping was repeated two days later.

### 3.2.5 Transient expression of proteins in *Nicotiana benthamiana*

For the transient expression of proteins in *Nicotiana benthamiana* a 4mL YEB- or LB-preculture with the respective selection was inoculated with one single colony of *A. thumefaciens* carrying the correct binary vector-DNA which should be transformed into the plant. The preculture was incubated shaking over night at 28°C. The 3mL LB- or YEB-main culture was inoculated with 0.5mL of the preculture and grown shaking for 4h at 28°C. The cells were pelletized by centrifugation with 4000rpm at 4°C for 15min and resuspended in precooled transformation solution. The volume of the transformation solution was calculated with 0.5mL transformation solution per leaf which should be infiltrated. When two or more constructs should be infiltrated at once, the resuspended *A.thumefaciens* cells transformed with the respective binary vector were mixed 1:1. Subsequent to the incubation of the cells on ice for at least 1h, the cells were injected into the tobacco leaf using a syringe without the needle. The samples were analyzed by microscopy two to three days after the transformation.

### 3.2.6 Induction of protein expression in *N. benthamiana* with β-estradiol

Transient expression of proteins in infiltrated leaves of *Nicotiana benthamiana* was induced 4-8h before the microscopic analysis. Therefore the induction solution was applied with a brush to the abaxial side of the leaves and incubated for 4-8h.

### 3.2.7 Yeast-two-hybrid

The Yeast-two-hybrid system was used to analyze physical *in vivo* protein-protein-interactions of cytosolic and nuclear proteins. For the interaction test fusion-contructs were generated fusing the proteins which should be tested for interaction to either the N-terminal GAL4-activating domain (AD) of the GAL4-transcription factor using the *pGADT7*-vector or the GAL4-DNA binding domain (BD) using the *pGBKT7*-vector. The yeast-two-hybrid system and the interaction test bases on the reconstitution of the GAL4-transcription factor upon the physical interaction of two proteins which then leads to the transcriptional activation and translation of the auxotrophy marker gene *ADE2* and therefore to the

ability of growth on ADE depleted medium. For the interaction test 4mL of CSM-Leu$^-$-Trp$^-$ were inoculated with three colonies of co-transformed pJ69-4A and grown over night at 28°C shaking. The cells were pelletized by centrifugation with 2000g for 10min and washed twice with sterile MilliQ water. Subsequently the cells were resuspended in sterile MilliQ water to $OD_{600}$=1.0 and a dilution series was generated with $OD_{600}$=0.1 and $OD_{600}$=0.01. 10µL of each dilution were dropped on the growth control medium CSM-Leu$^-$-Trp$^-$ and the auxotrophy-medium CSM-Leu$^-$-Trp$^-$-Ade$^-$. The growth at 28°C was documented for four to six days by scanning of the plates.

### 3.2.8 Mating-based split-ubiquitin screen

The mating-based split-ubiquitin system was used to analyze *in vivo* protein-protein-interactions of membrane-bound proteins or the interaction between a protein which is bound to a membrane (bait) and a cytosolic protein (prey). It utilizes ubiquitin which was split into two halves, the N- (Nub) and C-terminal (Cub) half. The N-terminal half was mutated to avoid spontaneous reassembling (NubA) and Cub was fused N-terminally with a transcriptional activator (*lexA-VP16*). For the protein-protein interaction test fusion constructs were generated, fusing a bait and a prey protein N-terminally to Cub (*pMetYC)* and NubA (*pXNubA22*). When the bait and prey protein interact, Cub and NubA are brought into close proximity and are hence recognized as a functional ubiquitin. This leads to the release of the lexA-VP16, therefore to the activation of the *Ade2*-, *His3*- and *lacZ*-gene and the loss of the Ade- and His-auxotrophy (Grefen *et al.*, 2009).

#### 3.2.8.1 Mating of Saccharomyces cerevisiae

For the protein-interaction test the transformed THY.AP4 and THY.AP5 expressing the proteins which should be tested had to be mated. Therefore over-night cultures with 4mL of the respective CSM-auxotrophy medium were inoculated with at least three colonies and grown at 28°C shaking. Cells of 2mL were pelletized using centrifugation with 1000g for 10min. The cells were resuspended in YPAD-medium whereat 20µL per mating were used.

20µL of bait and prey each were mixed in all combinations which should be tested for interaction. Subsequently 4µL of each mating were dropped on a YPAD-plate and grown over night at 28°C. The colonies of the mating were resuspended in 50µL of sterile MilliQ water respectively, 7µL each dropped on CSM-Ade$^+$-His$^+$ plates and grown for 1-3d at 28°C.

#### 3.2.8.2 Interaction test

4mL CSM-Ade$^+$-His$^+$ were inoculated with one colony of the mated *S. cerevisiae* strains respectively and grown over night at 28°C. 2mL of the culture were stored for the denaturing extraction of proteins. For the interaction test, cells of 100µL culture were pelletized using centrifugation with 1000g for 10min, washed three times with 100µL of sterile MilliQ water and resuspended in the volume of sterile MilliQ water for $OD_{600}$=1.0. A dilution series with $OD_{600}$=0.1 and $OD_{600}$=0.01 was generated. 5µL of each mating in each dilution was dropped on CSM minimal medium, CSM-Ade$^+$-His$^+$ as well as on CSM-Met$^+$ and grown at 28°C for up to 8d. The growth was monitored every day by scanning of the plates.

#### 3.2.9 Propidium iodide (PI) staining

Propidium iodide (PI) is a cationic dye that does not cross intact membranes but binds to cell walls, forming an outline of living cells. Seedlings and leaves were mounted in 0.1mg/mL PI on slides, incubated for at least 10min and then analyzed with the epifluorescence or confocal microscope. The excitation wavelength for PI is 536nm, the emission maximum at 617nm.

## 3.3 Physiological methods

#### 3.3.1 Seed surface sterilization

##### 3.3.1.1 Seed surface sterilization with sodium hypochlorite

At most 50mg of *Arabidopsis thaliana* seeds were surface sterilized with 1mL sodium hypochlorite solution for 5min and washed four times with 1mL of sterile 0.01% Triton-X-100.

##### 3.3.1.2 Seed surface sterilization with ethanol

At most 50mg of *Arabidopsis thaliana* seeds were surface sterilized with 1mL ethanol solution and shaken overhead for 15min. The ethanol solution was replaced by 100% ethanol and the seeds again shaken overhead for 15min at most. The seeds were pipetted on sterile filter paper, dried and stored for further use.

##### 3.3.1.3 Seed surface sterilization with chloric gas

At most 50mg seeds of *Arabidopsis thaliana* were placed in the desiccator. In a beaker glass set in the desiccator as well, 50mL 12% sodium hypochlorite and 1.5mL 37% HCl were mixed and the lid of the desiccator was immediately closed. The seeds were incubated in the formed chloric gas for three hours and were directly used or stored after the evaporation of the gas.

#### 3.3.2 Cultivation of *Arabidopsis thaliana*

For all physiological experiments seeds were used which originate from plants which were contemporaneously grown in the greenhouse.

##### 3.3.2.1 Cultivation of *Arabidopsis thaliana* on soil

To synchronize the germination of the seeds, surface sterilized *Arabidopsis thaliana* seeds were stratified in 1mL MilliQ water at 4°C for at least 24h. Then the seeds were pipetted on soil and the trays covered by a hood for the first week. Depending on the purpose the plants were grown in the greenhouse or the phytochambers.

For plants which should be used to isolate mesophyll protoplasts, surface sterilized *Arabidopsis thaliana* seeds were stratified in 1mL MilliQ water at 4°C for at least 24h and then pipetted on soil in 6cm pots. The tray, the pots were placed in, were covered by a hood for two weeks. After two weeks the seedlings were singularized in 6cm pots and grown for additional two weeks covered by a hood. Subsequently to one week of growth without a hood the plants could be used for mesophyll protoplast isolation.

#### 3.3.2.2 Cultivation of *Arabidopsis thaliana* on ½ MS-Agar plates

Surface sterilized *Arabidopsis thaliana* seeds were individually placed with autoclaved toothpicks on petri dishes with ½ MS-Agar supplemented with different substances. To synchronize the germination of the seeds, the dishes were incubated at 4°C for 1-4d.

#### 3.3.2.3 Cultivation of *Arabidopsis thaliana* in liquid culture

*Arabidopsis thaliana* seeds, which were surface sterilized with chloric gas, were stratified in 1mL sterile MilliQ water at 4°C for 3d. The MilliQ water was replaced by the growth medium and the seeds were pipetted into the growth vessel. They were cultivated at continuous light (40µmol $m^{-2}$ $s^{-1}$) shaking with 80u/min. After 10d the growth medium was renewed, after 14d the seedlings were treated and harvested respectively.

To obtain seedlings for the analysis of the phosphoproteome 20mg seeds of *ahk2 ahk3*, *ahk1-3* and their wildtypes Col-0 and Ws-2 were grown in 50mL JPL medium in 250mL Erlenmeyer flasks whereas Ws-2 and *ahk1-3* have been stratified in 5µM gibberellic acid (GA3). Col-0 and *ahk2 ahk3* grew at 22°C, Ws-2 and *ahk1-3* at 20°C. After 14d the media *ahk2 ahk3* and Col-0 were growing in were supplemented with kinetin to a final concentration of with 100ng/mL. Subsequent to 10min shaking at 80u/min in kinetin-supplemented media the seedlings were harvested, frozen in liquid nitrogen and stored at -80°C. In a control experiment (mock treatment) the seedlings were not treated with kinetin and harvested directly. For *ahk1-3* and its wildtype Ws-2 the 14d old seedlings were transferred into 50mL JPL medium respectively which was supplemented with 0.3M mannitol. Subsequent to 10min incubation in mannitol-supplemented media the seedlings were harvested, frozen in liquid nitrogen and stored at -80°C. In a control experiment (mock treatment) the seedlings were transferred to JPL medium without mannitol and harvested like the mannitol-treated seedlings.

For the analysis of the seedlings' sodium-, potassium- and calcium-content, 3-5mg seeds were stratified in 5µM gibberellic acid and grown in 2mL JPL medium in 24-well micro titer plates at 20°C. After 14d the seedlings were transferred into 2mL JPL medium which was supplemented with 0.3M mannitol. Subsequent to 10min incubation in mannitol-supplemented media the seedlings were harvested, frozen in liquid nitrogen and stored at -80°C. In a control experiment (mock treatment) the seedlings were transferred to JPL medium without mannitol and harvested like the mannitol-treated seedlings.

### 3.3.3 Cultivation of *Nicotiana benthamiana*

The seeds of *Nicotiana benthamiana* were sown on P-soil. Two week old seedlings were singularized into 6cm pots with the soil mixture and grown for additional two to three weeks at 23°C/20°C (day/night), 14h light and 60% humidity.

### 3.3.4 Crossing of Arabidopsis thaliana lines

Arabidopsis thaliana lines were cultivated on soil in the greenhouse as described until they were flowering. For the crossing of Arabidopsis thaliana lines, sepals, petals and stamen were removed from flowers which were going to open the next hours. The stigma of the carpel was then pollinated with pollen of the desired Arabidopsis thaliana line. All flowers which were not pollinated like this were removed from the plant. The seeds which resulted from this pollination were cultivated and genotyped

to analyze that both alleles were there. The plant lines were progenated like this until homozygote plant lines could be identified.

### 3.3.5 Destaining of *Arabidopsis thaliana* with acidified methanol

The plant material was put into acidified methanol and incubated for 20min at 55°C. The acidified methanol was replaced by the neutralization solution. After the destaining the plant material was rehydrated by replacing the neutralization solution by 40%, 20%, 10% ethanol and finally by $H_2O$.

### 3.3.6 Protoplast isolation

#### 3.3.6.1 Protoplast isolation of mesophyll cells

The isolation of mesophyll protoplasts was performed as described by Yoo *et al.*, 2007 and modified by Anna Jehle. Leaves were cut with a razor blade (0.5-1mm) and incubated in the enzyme solution (~10-20 leaves/5mL enzyme solution). Leaf strips were vacuum infiltrated in the dark for 30min using a desiccator and incubated in the dark for 3h at room temperature. Subsequently the enzyme/leaf solution was diluted with the same volume W5 solution, gently swirled to release the protoplasts from the leaf strips and filtered through a nylon mesh (75µm). Protoplasts were pelletized by centrifugation at 4°C with 200g for 1min in a round-bottomed tube (14mL, Roth) and resuspended in W5 whereas the double volume of initially used enzyme solution was utilized. Then the protoplasts were counted in a hemocytometer and incubated on ice for 30min to 3h. The protoplasts which settled on the bottom by gravity were resuspended in MMG solution to a final concentration of 200 000 protoplasts/mL.

#### 3.3.6.2 Protoplast isolation of seedlings

Hypocotyls and cotyledons of 4d old *Arabidopsis thaliana* seedlings grown on JPL medium plates were separated from roots, transferred to 1mL 300ME solution, cut into small pieces and incubated gently shaking for at least 3h in the dark. The isolated protoplasts were filtered through a 50µm nylon filter, harvested by centrifugation with 80g at 4°C for 5min and washed three times with ice-cold 300M solution. The protoplasts were resuspended in 0.5mL 300M and stored on ice for at least 30min before an experiment was started (Wu *et al.*, 2013).

### 3.3.7 Transformation of *Arabidopsis thaliana* mesophyll protoplasts

The transformation of *Arabidopsis thaliana* mesophyll protoplasts was performed as described by Yoo *et al.*, 2007 and modified by Anna Jehle. For the transformation in total 10µg plasmid DNA were gently mixed with 100µL aliquots comprising 20 000 protoplasts and 110µL of PEG solution. The mixture was incubated for 5min, subsequently diluted with 400µL W5-solution and centrifuged at 4°C with 200g for 1min. The pelletized protoplasts were resuspended in 100µL W5-solution supplemented with 200µM luciferin and distributed in aliquots of 100µL with 20 000 protoplasts per well in a 96-well plate. The protoplasts were incubated for 16h in the dark at room temperature. For transformation of several batches up-scaling of all components was performed whereas at least two technical replicates per transformation were used.

### 3.3.8 Protoplast Luciferase assay

After 16h of incubation, transformed *Arabidopsis thaliana* mesophyll protoplasts were treated by the addition of stock solutions in the same volumes and the luminescence of protoplast samples was quantified *in vivo* using a luminometer.

### 3.3.9 Protoplast swelling assay

For the protoplast swelling assay 20µL of the isolated protoplasts were added to approximately 200µL 300M solution in a perfusion chamber constructed by Gerhard Obermeyer placed under the microscope. Protoplasts were allowed to settle down for about 5-10min. The chamber was then perfused with 300M solution to select protoplasts sticking well to the glass bottom of the perfusion chamber. The perfusion solution was then changed to the hypo-osmolar 150M solution. Images of the swelling protoplasts were taken each second for at least 1min with a video-camera equipped microscope. The osmolality of all media was measured with a cryoscopic osmometer (Osmomat 030, Gonotec) and the following osmolalities were noted: 300M: 0.364osmol $kg^{-1}$; 150M: 0.207osmol $kg^{-1}$.
The video sequence was converted to an image sequence with one picture per second using STOIK Video Converter 3. For each time point the diameter and cross-section area of the protoplasts was measured using ImageJ and the volume as well as the surface area was calculated. The linear regression curve of the increase of the protoplast volume per time was used to calculate the water flux per $\mu m^2$ protoplast membrane which was defined as water flux density.

### 3.3.10 Pathogen assay with *Alternaria brassicicola* (by Jens Riexinger and Birgit Kemmerling)

*Arabidopsis thaliana* lines were cultivated on soil in the short day chamber as described. Three week old plants were watered properly prior to the assay whereas the leaves were kept dry. The plant lines were mixed in the trays and two leaves per plant labeled. The glycerol stock of $2x10^7$ spores/mL was diluted with sterile water to $1x10^6$ spores/mL and kept at room temperature. Two to six droplets of 5µL spore solution were put on the leaves. Bonitation was executed after 7, 10 and 13 days after infection using the bonitation code which is shown in fig. 3.2.

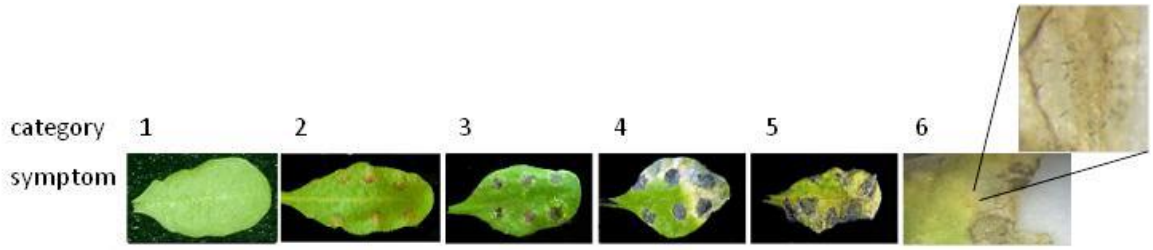



figure 3.2: Bonitation code for the pathogen assay with *Alternaria brassicicola*
According to the severity of symptoms the infected leaves show, they are classified into six categories: category1: leaf shows no symptoms; category2: light brown spots at infection site; category3: dark brown spots at the infection site; category4: spreading necrosis; category5: leaf maceration; category6: sporulation.

Category one means that the leaf does not show any symptoms, category two that the leaf shows light brown spots at the infection site, category three that the leaf shows dark brown spots at the infection site, category four describes spreading necrosis, category five leaf maceration and category six sporulation.

Trypan blue was used to selectively stain dead tissue. Therefore infected leaves were put into a 6-well micro titer plate filled with 2mL Trypan blue staining solution per well and boiled for 45-60s in a 100°C water bath. Subsequently to the incubation for 5min at room temperature, the staining solution was replaced by 1.5mL chloral hydrate solution and incubated for 6h. After 6h the chloral hydrate solution was renewed. After incubation in chloral hydrate over night the leaves were put into 20% glycerol and analyzed by microscopy.

### 3.3.11 Salt response assay (by Christa Testerink)

The response of Ws-2, *ahk1-3* and *ahk1-4* to 75mM NaCl was executed and analyzed like described in Julkowska *et al.*, 2014.

### 3.3.12 Determination of flowering time

According to Kang *et al.*, 2015, flowering time was measured as the number of days from seed sowing to the opening of the first flower. The *Arabidopsis thaliana* plants were cultivated as described in the long day and short day phytochamber.

### 3.3.13 Germination assay

Surface sterilized seeds were individually placed with autoclaved toothpicks on half strength MS salts, 2mM MES and 1% phytoagar as well as on media supplemented with 0.3M mannitol, stratified for 4d at 4°C to synchronize the germination and grown for six days at continuous light conditions at 20°C. Germination was monitored every day by scanning of the plates and counting of germinated seeds. The germination rate was defined as the percentage of seeds which have germinated after five days of continuous light. The germination time was defined as the mean value of days the seeds needed to germinate. The experiment was carried out in at least three biological replicates with 50 seeds per line and treatment respectively. Data were averaged between these replicates.

### 3.3.14 Hydrotropic growth assay

Surface sterilized seeds were individually placed with autoclaved toothpicks on ½ MS-agar, stratified at 4°C for 3d to synchronize germination and grown in constant light conditions at 20°C for 4d. To test the hydrotropic response plates were needed with a diagonal gradient of water potential ($\psi_W$). To obtain this, 65mL half strength MS salts with 1% phytoagar were casted into 12cm x 12cm petri dishes. Once solid, the half strength MS-agar was diagonally cut with a sterile blade of a scalpel and the lower part removed. The lower part was filled with 30mL half strength MS-agar supplemented with 400mM sorbitol. The 4d old seedlings were transferred to these plates orienting their roots straight and vertical to the bottom frame of the petri dish and placing their root tips precisely on a parallel line of 3mm distance to the medium interface. The plates were put in an upright position to constant light. After at least 12h of growth the hydrotropic response of the different plant lines was analyzed using the growth angle α of the roots in reference to the gravitropic growth stimulus (g) as indicator. The angle was measured using ImageJ. The experiment was repeated more than three times with 35 seedlings per line respectively. Data were averaged between these replicates.

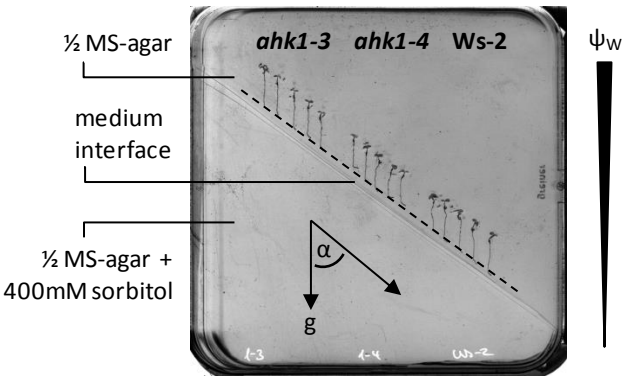



figure 3.3: Experimental setup to test the hydrotropic response.
4d old seedlings of different *Arabidopsis thaliana* lines (here: Ws-2, *ahk1-3*, *ahk1-4*) were transferred to plates which were splitted diagonally in an upper part with ½ MS-Agar and a lower part with ½ MS-Agar supplemented with 400mM sorbitol whereat the root tips were precisely placed on a line lying 3mm distant parallel to the medium interface in the upper part (dashed line). This leads to a diagonal gradient of water potential ($\psi_W$). To analyze the hydrotropic response of the different plant lines the growth angle α of the roots in reference to the gravity (g) was used as indicator which was measured with the use of ImageJ.

### 3.3.15 Gravitropic growth assay

Surface sterilized seeds were individually placed on ½ MS-agar and ½ MS-agar supplemented with 1µM methyl-jasmonate (MeJA), stratified at 4°C for 3d to synchronize germination and grown in constant light conditions at 20°C for 3d whereat the plates were put into an upright position. To investigate the gravitropic response of root growth, the plates were turned for 90° to the left. After 2d of additional growth the plates were scanned for the analysis. Using ImageJ the growth angle α of the root towards the applied gravitropic stimulus in reference to the original direction of gravity was analyzed and defined as curvature. The experiment was executed once with at least 90 seedlings per line and treatment.

### 3.3.16 Investigation of lateral root development

Lateral root development is favored at the bending zone of main roots after an altered gravitropic stimulus. Stage I lateral root primordia were usually first detected 18h after gravitropic induction. The formation of each subsequent stage usually takes 3h (Péret *et al.*, 2012). Therefore the bending point of gravitropically induced roots was analyzed and the lateral root primordia categorized 18h and 42h after the gravitropic induction of 3d old seedlings. To obtain these seedlings, at least 40 surface sterilized seeds per line were individually placed on ½ MS-agar. The seeds on the plates were stratified for 3d at 4°C to synchronize germination and grown for 3d in an upright position in constant light conditions at 20°C. The same seed set was sown for the 18h time point as well as for the 42h time point. The gravitropic induction was applied by the 90° turn of the plate to the left. 18h and 42h after the gravitropic induction the seedlings were transferred into a 24-well micro titer plate and destained with acidified methanol. Subsequently the seedlings were mounted on glass slides in 50% glycerol and incubated over night. With the use of differential interference contrast microscopy the lateral root primordia were categorized into the different stages. The number of plants with a primordium in the respective stage was determined and the percental amount of plants in regard to the total number of

analyzed plants at the particular time point calculated. The experiment was repeated two times. Data were averaged between these replicates.

### 3.3.17 Analysis of stomatal density and stomatal index

Surface sterilized seeds were individually placed on half strength MS salts with 2mM MES and 1% phytoagar, stratified for 3d and grown at constant light at 20°C whereat the plates were put in an upright position. When the seedlings began to develop their first leaf they were transferred to half strength MS salts with 2mM MES and 1% phytoagar or half strength MS salts with 2mM MES and 1% phytoagar supplemented with 0.3M mannitol respectively.

The first leaves were stained with propidium iodide and imaged with the use of a confokal laser microscope (Leica, SP2).

Additionally surface sterilized seeds were individually placed on ½ MS-agar, stratified for 3d and grown for 3d at constant light at 20°C whereat the plates were put in an upright position. The cotyledons were stained with propidium iodide and imaged with the use of a confokal laser microscope (Leica, SP2).

The images of the outlined cells were merged with the use of ImageJ. For the analysis of stomatal density and stomatal index, the outlines of the cells had to be retraced. This was executed manually with PicsArt. Then the number of cells was counted and the stomatal density and stomatal index calculated.

### 3.3.18 Root growth assay

Surface sterilized seeds were individually placed on half strength MS salts with 2mM MES and 1% phytoagar or ½ MS-agar respectively. The seeds on the plates were stratified at 4°C for 3d to synchronize germination and were the grown at constant light and 20°C for 4d whereat the plates were put in an upright position. The 4d old seedlings were then transferred to osmotic stress media or media supplemented with hormones, inhibitors of hormone biosynthesis or signaling as well as on pathogen-associated molecular pattern (PAMPs). For each treatment a respective control was included. 20 seedlings per line and treatment were transferred. The treatment plates were scanned every day at the same time beginning from the day of transfer (“d0”) until eight days after the transfer (“d8”). For the analysis of root elongation, an overlay of the images of d8, d4 and d0 was generated. ImageJ was used to measure the root length of d0, d4 and d8 in one run. From these data, the total root length could be compared as well as the mean values of total root elongation which were determined like it was described by Kumar *et al.,* 2013. According to Wohlbach *et al.*, 2008 the mean percentage of root elongation based on the mean value of non-stressed control roots was calculated in addition to compare the results of both ways of analysis. To illustrate differences of several experiments in regard to root length and root elongation the mean percentage of root elongation based on the respectively treated wildtype root was calculated. For each type of analysis data were averaged between replicates when more than one replicate was executed.

### 3.3.19 Study of Arabidopsis thaliana seed size

At least 80 seeds per Arabidopsis thaliana line were filled in a petri dish which was scanned with 300dpi resolution. With the use of ImageJ binary pictures were generated and the seed size was measured using the tool “Analyze Particles”. Three seed batches were analyzed. Each seed batch

originated from plants which grew contemporaneously in the greenhouse. Data were averaged between these replicates.

### 3.3.20 Investigation of skotomorphogenesis

50 surface sterilized seeds per *Arabidopsis thaliana* line and treatment were individually placed on ½ MS-agar without supplementation, with supplementation of PAMPs, hormones, inhibitors of hormone biosynthesis and signaling as well as on osmotic stress media including the respective control. The seeds on the plates were stratified at 4°C for 3d to synchronize germination. 2h light at 20°C induced germination. Subsequently to the 2h of light induction the plates were carefully darkened by wrapping in aluminum foil and placed in an upright position in the constant light at 20°C. After 3d of growth in the dark the plates with the skotomorphogenic seedlings were scanned. With the use of ImageJ the hypocotyl and root length of the seedlings was measured. To illustrate the constant difference in length the mean percentage of hypocotyl and root length in reference to the respective wildtype was calculated. Data were averaged between replicates when more than one replicate was executed.

## 3.4 Biochemical methods

### 3.4.1 Agarose gel electrophoresis

In agarose gel electrophoresis DNA is separated according to its size by an electrical field. Depending on the size of the expected DNA fragments an agarose concentration of 0.8-3% (w/v) was chosen. Agarose was dissolved in 1X TAE buffer by boiling. After chilling to ~50°C the DNA stain ethidium bromide or Midori Green was added to a final concentration of 0.5µg/mL or 0.02% (v/v) Midori Green Advanced DNA Stain (Nippon Genetics Europe) and the mixture was poured into the electrophoresis cell. 1X TAE buffer was used as running buffer. DNA-samples and a size standard were mixed with DNA loading buffer in a ratio of 1:4 and loaded onto the gel. The gel was run with 5V/cm. The size of DNA fragments was reported using UV-light.

### 3.4.2 Extraction of DNA-fragments from agarose gels

The extraction of DNA-fragments from agarose gels was executed with the use of the Quick Gel Extraction Kit (invitrogen) and Gel Extraction Kit (genaxxon) according to the manuals.

### 3.4.3 Measurement of nucleic acid concentrations in solutions

The concentration of nucleic acids which were dissolved in 10mM Tris/HCl pH 8.0 or sterile MilliQ water was photometrically determined using NanoDrop1000 Spectrophotometer (Thermo Scientific). $OD_{260}$=1.0 correlates to a nucleic acid concentration of 50µg/mL (Sambrook *et al.*, 2001). The ratio of sample absorbance at 260nm and 280nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as “pure” for DNA, a ratio of ~2.0 is generally accepted as “pure” for RNA. A ratio which is lower indicates the presence of protein. The ratio of sample absorbance at 260nm and 230nm is an additional measure of nucleic acid purity whereas a ratio between 1.8 and 2.2 indicates “pure” nucleic acids.

### 3.4.4 DNA-sequencing

Sequencing of vector DNA was executed by GATC Biotech AG, D.

### 3.4.5 SDS-Polyacrylamid-Gel-Electrophoresis (SDS-PAGE)

SDS-Polyacrylamid-Gel-Electrophoresis (SDS-PAGE) was utilized to separate proteins according to their size. Therefore the electrophoresis chambers of BioRad were used. Proteins were extracted and loaded on the SDS-gel in Lyse and Load buffer. The Spectra™ Multicolor Broad Range Protein Ladder (Thermo Scientific) was used as marker for the protein size. SDS buffer was utilized as running buffer. The gel was run with 120V and 10-15mA/gel.

### 3.4.6 Coomassie staining

For the detection of proteins in the SDS-gel after the SDS-PAGE the gel was incubated shaking over night in Coomassie staining solution. Then the Coomassie staining solution was replaced by the destainer solution and the gels were incubated shaking until the protein bands were visible. For documentation, stained SDS-gels were then placed between two Cellophan (Roth) foils which were wetted with 20% glycerol, dried and scanned.

### 3.4.7 Western Blot

The proteins which were separated according to their size by SDS-PAGE were transferred by a Western wet Blot to an immobilizing PVDF-membrane (Millipore). The wet blot setup was performed in the following sequence: Anode, sponge, Whatman-paper (GE Healthcare), PVDF-membrane, SDS-gel, Whatman-paper, sponge, cathode in transfer buffer. Previously the PVDF-membrane was equilibrated in 100% methanol. The transfer was executed at 4°C using the electrophoresis chambers of BioRad with a constant current of 60mA over night or a constant current of 300mA for 1.5h.

### 3.4.8 Immunodetection

The proteins which were transferred and immobilized on the PVDF-membrane were detected with specific antibodies. After the Western wet Blot, the PVDF-membrane with the bound proteins was incubated in blocking solution for at least 1h at room temperature or over night at 4°C. Subsequently to three washing steps with TBS-Tween for 10min each the first antibody was added and incubated for at least 2h at room temperature or over night at 4°C. The first antibody binds specifically to the protein which should be detected. Excessive antibody was removed in three washing steps with TBS-Tween for 10min each. The second antibody which binds the first antibody and which is fused to the Alkaline Phosphatase (AP) was added and incubated for at least 1h at room temperature. Excessive antibody was again removed by three washing steps with TBS-Tween for 10min each. Subsequently to the equilibration of the PVDF-membrane in staining buffer for 5min the staining with the staining solution was executed. The staining solution contains 5-Bromo-4-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium (NBT). BCIP is oxidized by the AP to a blue indigo-dye whereas the BCIP oxidation leads to the reduction of NBT and therefore to the generation of a blue formazan-dye. The staining reaction was stopped by washing with MilliQ water as soon as clear band were visible. For documentation the PVDF-membrane was dried and scanned. Alternatively to the use of two antibodies just one antibody was applied when it was specifically binding to the tag and already fused to the AP.

### 3.4.9 Extraction of ions from dried plant material

To obtain dry plant material, the plant material of which the fresh weight was determined was wrapped in aluminum foil and dried at 80°C for several days. The dry weight was determined and the plant material transferred to 1.5mL reaction tubes. Glass beads of 1.25-1.65mm were added and the plant material grinded. 1mL 1M $HNO_3$ was added per 0.3g fresh weight. The samples were incubated over night at room temperature. Plant fragments were removed by centrifugation with 13000rpm for 1min.

### 3.4.10 Flame photometric measurement of $Na^+$-, $K^+$- and $Ca^{2+}$-concentration

As described on www.sherwood-scientific.com compounds of the alkali and alkaline metals are thermally dissociated into atoms at the temperature of a Bunsen burner flame and some of these atoms are further excited to a higher energy level. When these “excited” atoms return to the ground state, they emit radiation, which for the elements of these two groups lies mainly in the visible region of the electromagnetic spectrum. The wavelength of the light emitted from the flame is characteristic for the particular element. The intensity of this light is mainly proportional to the absolute quantity of the species present in the flame. The emitted radiation is isolated by an optical filter and then converted to an electrical signal by the photo detector.

For the measurement of the concentration of $Na^+$, $K^+$ and $Ca^{2+}$ which were extracted from dried plant material with 1M $HNO_3$ the extracts were diluted in the ratio 1:20. Before the ion concentration in the samples could be determined a respective reference curve was generated. For potassium-ions a reference curve was generated using KCl solutions in the concentrations 100µM, 200µM, 500µM, 1000µM and 2000µM. For sodium- and calcium-ions reference curves were generated using NaCl or $CaCl_2$ solutions respectively in the concentrations 20µM, 50µM, 100µM, 200µM and 500µM. As the optical filter has to be changed for each metal the generation of the reference curve and the measurement of the respective ion-concentration in the samples were executed consecutively.

The reference curves provided the relationship between the ion-concentration and the displayed value of the Sherwood flame photometer (model 410) for the different ions. Using the formula of the regression line the displayed values were calculated to the respective ion-concentration. The ion-concentration was normalized to the dry weight of plant material. 9-12 biological replicates per *Arabidopsis thaliana* line and treatment which were grown contemporaneously were measured and data averaged.

## 3.5 Bioinformatical methods

### 3.5.1 Prediction of protein domains

For the analysis of a protein’s amino acid sequence in regard to putative domains like transmembrane or kinase domains the amino acid sequence in the FASTA-format was submitted to elm.eu.org for domain prediction.

### 3.5.2 Search for similar sequences and phylogenetic analysis

To identify proteins with a similar nucleotide or amino acid sequence like AHK1 different BLASTs were executed using https://blast.ncbi.nlm.nih.gov/Blast.cgi. The AHK1 nucleotide sequence in FASTA-format was used as query sequence for blastn. Somewhat similar sequences were searched in the

databases “Nucleotide collection (nr/nt)”, “Expressed sequence tags (est)”, “Genomic survey sequences (gss)”, and “Whole-genome shotgun contigs (wgs)” for the respective organism. The AHK1 full length amino acid sequence as well as the amino acid sequence of the AHK1 extracellular domain (AHK1-ED) was used as query to run blastp and tblastn searching in the previously described databases for the respective organisms.

For the phylogenetic analysis of AHK1-ED the identified sequences were all translated to their amino acid sequence. For all amino acid sequences a prediction of protein domains was executed. Subsequently the sequences were reduced to their predicted transmembrane domains including their extracellular domain. These sequences were then aligned with the CLUSTAL multiple sequence alignment tool MUSCLE 3.8. The output is the sequence alignment as well as a phylogenetic tree.

### 3.5.3 Modeling of the AHK1-ED structure (by Michael Hothorn)

The modeling of the AHK1 extracellular domain (AHK1-ED) is based on the structure of the sensor domain of AHK4 (pdb id: 3T4J). The homology model of AHK1-ED was modeled with “Modeller” (https://salilab.org/modeller/) according to an alignment which was generated with “HHPRED” (https://toolkit.tuebingen.mpg.de/hhpred/).

# 4 RESULTS

During my Ph.D. thesis I was working on the molecular characterization of the *Arabidopsis thaliana* histidine kinase 1 (AHK1). This work can be divided into different parts, comprising tissue-specificity of AHK1 expression and the subcellular localization and dynamics of AHK1, the evolutionary conservation of the AHK1 extracellular domain (ED) and steps towards the elucidation of its structure and the identification of its perceived signal, the finding of conditions when *ahk1* knock down mutants show a reproducible phenotype in comparison to the wildtype as well as the analysis of AHK1-dependent changes in the phosphoproteome and the study of the molecular mechanisms of AHK1 signaling.

Additionally I have contributed to a phosphoproteomic study in which it was investigated which influence short term kinetin treatment has on the phosphoproteome in the wildtype Col-0 as well as in the cytokinin-receptor knock down mutant *ahk2 ahk3*.

The molecular characterization of AHK1 as well as the study of the phosphoproteome of *ahk2 ahk3* after short term kinetin treatment allows new insights into the network of hormones, signaling pathways and mechanisms of their regulation.

## 4.1 Molecular characterization of AHK1

### 4.1.1 Expression and subcellular localization of AHK1

#### 4.1.1.1 AHK1 expression

In the study of Urao *et al.* (1999) GUS-stained stable *Arabidopsis thaliana* lines containing an *AHK1-promoter β-glucuronidase* (*GUS*) fusion construct showed that the *AHK1-promoter* mediates strong GUS-activity in leaf bases and roots of rosette plants when the plants were exposed to either high- or low-osmolarity solutions but just weak GUS-activity when the plants were exposed to isotonic solutions or when the plants were untreated. Together with RNA gel blot analysis they could draw conclusions about rough localization of *AHK1* expression and semi-quantitative promoter activity.

To address the question about tissue-specificity of *AHK1-promoter* activity and to have the opportunity to investigate it in vital plants the construct of Katharina Caesar, in which 2074bp upstream of *AHK1*'s ATG is fused as promoter ($AHK1_{pro}$) to a *mCherry* which is linked to a *nuclear localization signal* (*NLS*) to concentrate the signal of low expression levels (fig. 4.1 A), was stably transformed into *Arabidopsis thaliana* Col-0 ecotype and progenated until homozygous lines could be obtained.

The analysis of this line revealed different $AHK1_{pro}$ activity in light-grown and etiolated seedlings. In three day old light-grown seedlings $AHK1_{pro}$ activity could be detected in the cotyledons, in the vascular tissue of the lower part of the hypocotyl as well as in the vascular tissue of the differentiation zone of the root. In the meristematic, the transition and elongation zone of the root no $AHK1_{pro}$ activity

could be revealed by nuclear mCherry signal (fig. 4.1 B). In three day old etiolated seedlings *AHK1*$_{pro}$ activity could be revealed in the cotyledons and in the vascular tissue of the hypocotyl and of the root. Thereby it has to be pointed out, that the section of the hypocotyl in which *AHK1*$_{pro}$ activity could be detected is much bigger in etiolated seedlings than in light-grown seedlings and that the part of the root in which *AHK1*$_{pro}$ activity could be shown is bigger in light-grown seedlings. When the seedlings were grown on different concentrations of mannitol, light-grown as well as etiolated seedlings still show *AHK1*$_{pro}$ activity in the cotyledons and in the vascular tissue in the same sections of the hypocotyl and root as in the control samples (appendix A10, A11).

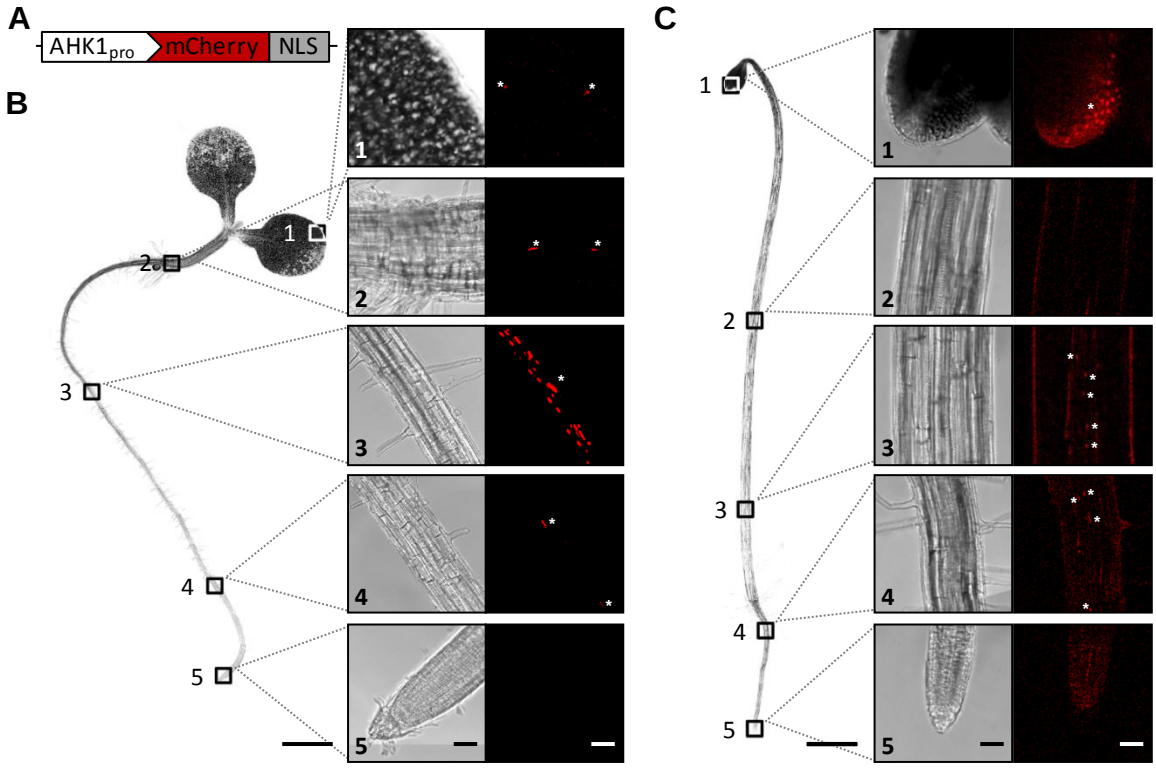



figure 4.1: Activity of the *AHK1-promoter* in Col-0 seedlings.
(A) To analyze the tissue specificity of the *AHK1-promoter* (*AHK1*$_{pro}$, white) it was fused to *mCherry* (red) and a *nuclear localization signal* (*NLS*, grey) in a binary plant vector and stably transformed into *Arabidopsis thaliana* Col-0. The tissue specificity of *AHK1*$_{pro}$ activity and mCherry expression was analyzed in three day old light-grown seedlings (B) and three day old etiolated seedlings (C) with confokal microscopy. The single pictures were assembled to obtain a whole picture of the whole seedling. Shown are brightfield overview images of the seedlings as well as close ups (1-5) which show brightfield images and mCherry signal (red). The bars in the overviews give 1mm, the bars in the close ups 0.05mm. Stars mark nucleic mCherry signal.

#### 4.1.1.2 Subcellular localization of AHK1

Katharina Caesar could show in previous studies that AHK1 localizes to the plasma membrane and to vesicle-like structures. To clarify the identity of these vesicle-like structures *AHK1-GFP* was transiently co-expresssed under the control of the *CaMV 35S*- (Odell *et al.* 1985) or the β-estradiol inducible promoter *lexA*$^{-46}$*35S* (Zuo *et al.* 2000) together with endosomal markers which were described in Nelson *et al.* (2007) or obtained by Peter Pimpl in *N. benthamiana*. AHK1-GFP did neither co-localize with the peroxisomal marker CD3-983 px-rk (fig. 4.2 A), GOT1-mCherry as marker for the golgi (fig. 4.2

C) nor VTI12-mCherry as marker for the trans-golgi-network and early endosomes (fig. 4.2 D). Co-localization could be revealed for AHK1-GFP and CD3-967 g-rk as golgi marker (fig. 4.2 B), RabD2a-mCherry as marker for endosomes and the golgi-system (fig. 4.2 E), ARA6-mCherry as marker for late endosomes and prevacuolar compartments (fig. 4.2 F), PEP12-mCherry as marker for the post-golgi compartment (fig. 4.2 G) and with RabA5d-mCherry as marker for the recycling endosome (fig. 4.2 H).

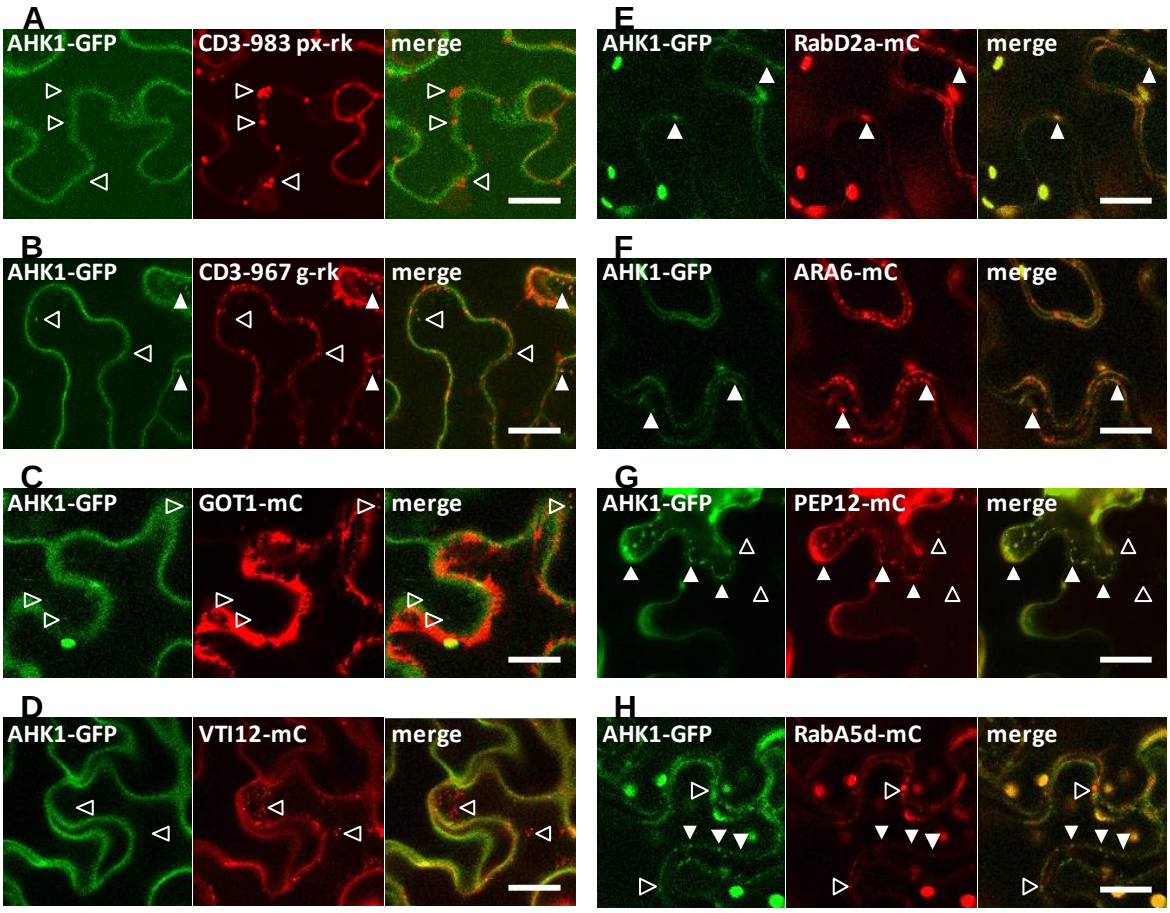



figure 4.2: Subcellular localization of AHK1-GFP
Transient expression of *AHK1-GFP* with (A) the peroxisomal marker *CD3-983 px-rk*; (B) the golgi-marker *CD3-967 g-rk*; (C) the golgi-marker *GOT1-mC*; (D) *VTI12-mC* as marker for the trans-golgi-network and early endosomes; (E) *RabD2a-mC* as marker for endosomes and the golgi-system; (F) *ARA6-mC* as marker for late endosomes and prevacuolar compartments; (G) *PEP12-mC* as marker for the post golgi compartment and with (H) *RabA5d-mC* as marker for the recycling endosome. (A, B) were obtained from Andreas Nebenführ. The markers are fused with RFP and under the control of the *CaMV 35S-promoter*. These marker constructs were co-transformed into *N. benthamiana* with *AHK1-GFP* is under control of the *CaMV 35S-promoter* (*pH7FWG2-AHK1*). (C-H) The markers for organelles fused with *mCherry* (*mC*) were obtained from Peter Pimpl who used the β-estradiol inducible promoter *lexA*$^{-46}$*35S* (Zuo *et al.* 2000). These marker constructs were co-transformed with *AHK1-GFP* under control of the *lexA*$^{-46}$*35S* promoter (*pABind-AHK1-GFP*). The subcellular localization was analyzed two days after transformation (A-H) and 4-24h after β-estradiol induction (C-H). The tobacco leaves were mounted in water. White outlined arrows mark vesicle-like structures which do not show co-localization, white arrows mark co-localization. The scale in all images is 20µm.

The same localization pattern could be observed after mounting of the tobacco leaf discs in 0.8M mannitol except for GOT1-mCherry, which in contrast to mounting in water shows co-localization with

AHK1-GFP and RabA5d-mCherry which did not show co-localization any more (appendix A12). AHK1-GFP did neither localize to the endoplasmic reticulum, mitochondria nor plastids (appendix A13).

### 4.1.2 Signal perception of AHK1

AHK1 was suggested to be a mechano-sensitive osmosensor but nothing was really known about the mechanisms of the perception of osmotic stress. To get a hint about the mechanism, the extracellular domain of AHK1 (AHK1-ED) as putative site of signal perception, comprising amino acid 100-446, was analyzed in regard to the evolutionary conservation of the amino acid sequence as well as in structure.

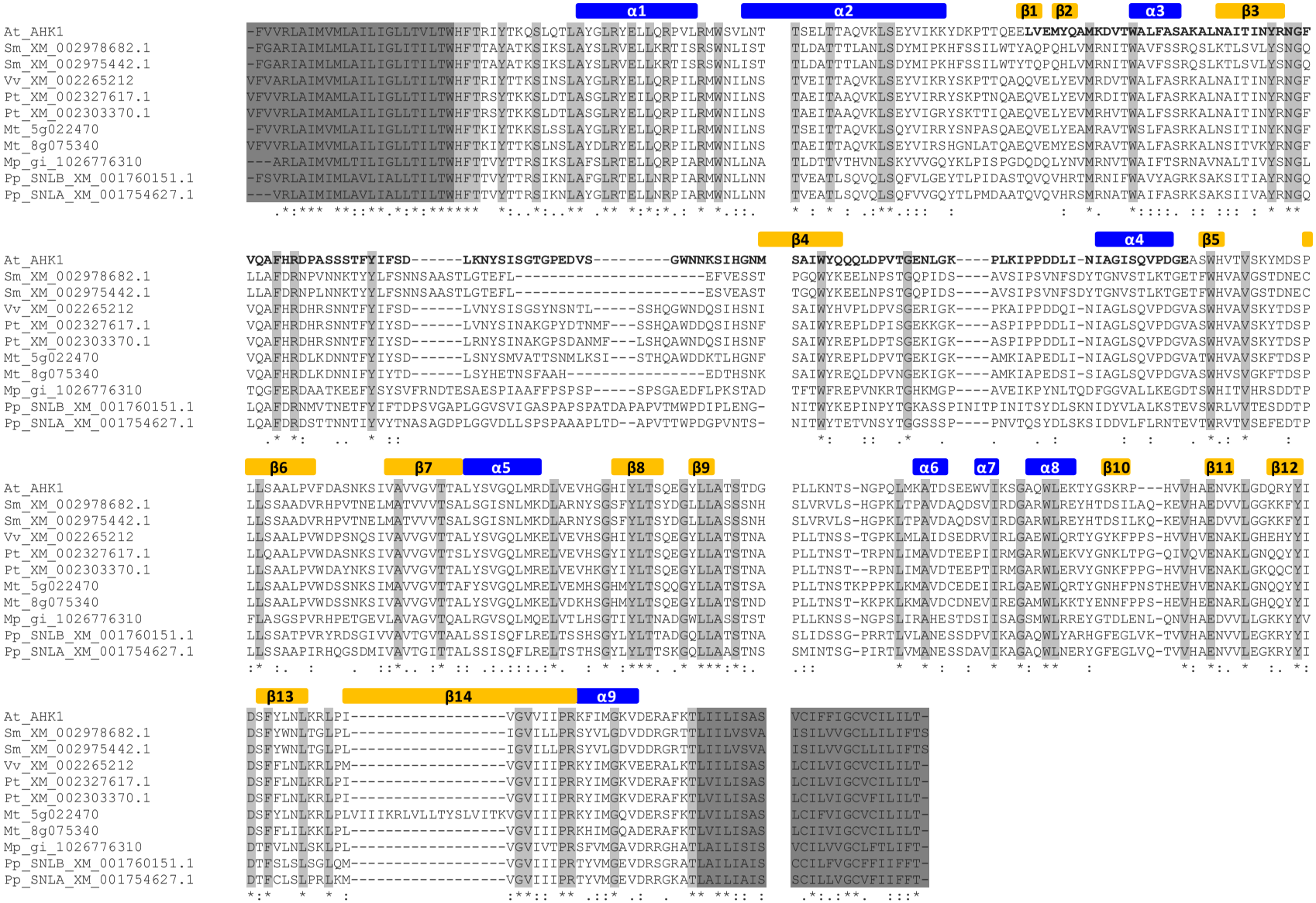
#### 4.1.2.1 Evolutionary conservation of the extracellular domain of AHK1

Urao *et al.* (1999) revealed that in *Arabidopsis thaliana* AHK1 shows the highest degree of similarity to CKI1 and that the histidine kinase and receiver domain show high similarity with SLN1 in *Saccharomyces cerevisiae*. As in other sensor hybrid histidine kinases like the cytokinin receptors AHK2, AHK3 and AHK4 the signal is perceived with the use of the CHASE domain which is located between the two transmembrane domains (figure 4.12) an importance of the extracellular domain of AHK1 (AHK1-ED) for signal perception was suggested. Different sequence alignments of the sequences which are located between the two transmembrane domains revealed that the sequence similarity of SLN1 to CKI1 (appendix A15) is bigger than to AHK1 (appendix A16) and the sequence similarity of AHK1 to CKI1 (appendix A17) is bigger than to SLN1 (appendix A16) indicating that AHK1 might not be an ortholog of SLN1. An alignment of the domain between the extracellular domains of AHK1, CKI1 and SLN1 is shown in appendix A18 and did not show an increased similarity. In an additional analysis of sequence similarities the amino acid sequence of AHK1-ED including the predicted transmembrane domains (aa 77-99, aa 447-469) was used as query to run blastp and tblastn against different species.

In *Aquilegia coerulea*, *Coleochaete orbicularis*, *Cyanophora paradoxa*, *Oryza sativa*, *Picea abies*, *Saccharomyces cerevisiae*, *Spirogyra pratensis* and *Zea mays* there were no sequences found which are similar to AHK1 but in *Marchantia polymorpha* (Mp), *Medicago truncatula* (Mt), *Physcomitrella patens* (Pp), *Populus trichocarpa* (Pt), *Selaginella moellendorffii* (Sm), and *Vitis vinifera* (Vv).

With the CLUSTAL multiple sequence alignment tool MUSCLE 3.8 it could be shown that 63 (18.15%) of the 347 amino acids of the AHK-ED without the transmembrane domains and its similar sequences are identical and 71 (20.46%) are highly conserved from moss and moss fern up to the dicotyledon tree *Populus trichocarpa*.

figure 4.3: (next side): Conservation of the extracellular domain of AHK1 (AHK1-ED)
CLUSTAL multiple sequence alignment by MUSCLE (3.8) of the amino acid sequences of *Arabidopsis thaliana* (At) AHK1-ED (aa100-446) and similar sequences from *Selaginella moellendorffii* (Sm), *Physcomitrella patens* (Pp), *Marchantia polymorpha* (Mp), *Vitis vinifera* (Vv), *Medicago truncatula* (Mt) and *Populus trichocarpa* (Pt). Predicted transmembrane domains (AHK1-ED: aa77-99; aa447-469, dark grey) were included in the alignment. Identical residues (*) are shaded in grey. Highly conserved residues are designated with ":", weakly conserved residues with ".". The sequence of the PAS-domain in AHK1-ED is marked with fat letters. Sequences which, according to the homology model of Michael Hothorn, putatively form α-helices or β-sheets are highlighted above the sequences in blue and yellow.


α1
α2
β1
β2
α3
β3
β4
α4
β5
β6
β7
α5
β8
β9
α6
α7
α8
β10
β11
β12
β13
β14
α9
At_AHK1
Sm_XM_002978682.1
Sm_XM_002975442.1
Vv_XM_002265212
Pt_XM_002327617.1
Pt_XM_002303370.1
Mt_5g022470
Mt_8g075340
Mp_gi_1026776310
Pp_SNLB_XM_001760151.1
Pp_SNLA_XM_001754627.1

At least 38 (10.95%) amino acid residues could be identified as weakly conserved (fig. 4.3). 39 (61.90%) of the identical amino acid residues are nonpolar, 15 (23.80%) are polar, 6 (9.52%) are basic and 3 (4.76%) are acidic. The predicted Per-Arnt-Sim (PAS) domain which consists of 119 amino acid residues of AHK1-ED comprises 10 of in total 63 amino acid residues which are identical in all sequences which are similar to AHK1-ED, 20 of 71 which are highly conserved and 9 of 38 which are weakly conserved. The average size of the identified sequences with similarity to AHK1-ED is 355 amino acids with 10 amino acids more or less whereas the putative extracellular domain of SLN1 comprises 288 amino acids. The inclusion of SLN1 to the multiple sequence alignment with the AHK1-ED orthologs leads to a massive reduction in conserved residues (appendix A14) which implicates that SLN1 confirms the finding that SLN1 might not be an AHK1 ortholog.

#### 4.1.2.2 Homology model of the extracellular domain of AHK1

Based upon just 13% sequence similarity to AHK4, Michael Hothorn could derive a homology model for the structure of the extracellular domain of AHK1 (fig. 4.4).

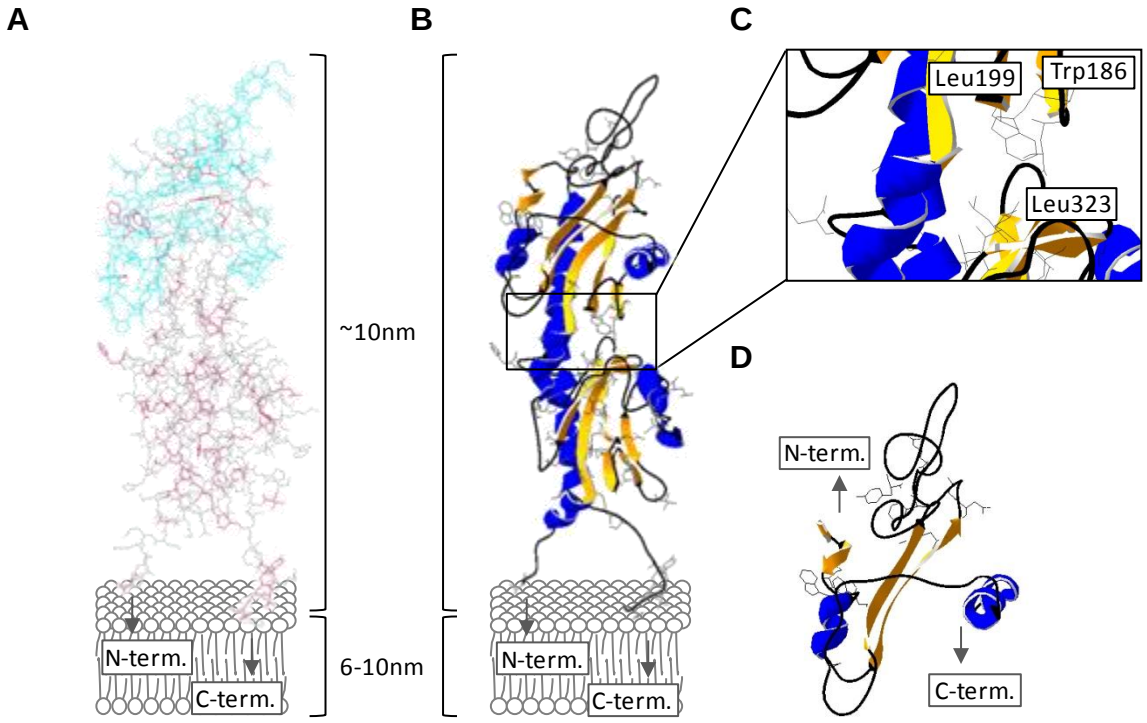



figure 4.4: Homology model of the extracellular domain of AHK1
The construction of the homology model of the extracellular domain of AHK1 (AHK1-ED; amino acid 101-447) is based on the 13% sequence similarity of AHK1 and AHK4. (A) shows the tertiary structure of AHK1-ED. The predicted PAS domain is highlighted in turquoise and the residues which are identical in all of the identified similar sequences of AHK1 are assigned in red. (B) The tertiary structure shows a combination of α-helices (blue) and β-sheets (yellow). The depicted amino acid residues are those which are identified in all sequences similar to AHK1-ED. (C) Close up of the „neck“ region of AHK1-ED. (D) Tertiary structure of the PAS domain including α-helices (blue) and β-sheets (yellow) comprising the identical residues of the similar sequences of AHK1-ED.

The model shows the amino acid sequence starting with aa100 of AHK1 full-length protein after the first predicted transmembrane domain reaching to aa440 which is the aa before the second predicted transmembrane domain. Starting from aa100, the sequence first forms two α-helices which build a kind

of backbone for the AHK1-ED. At the plasma membrane distal end of this α-helix backbone the predicted PAS domain (aa164-282) is formed (fig. 4.4 A, turquoise) comprising two α-helical structures as well as four β-sheets (fig. 4.4 D). Three additional β-sheets are involved in this distal part of the AHK1-ED. The plasma membrane proximal part of the AHK1-ED is formed by five α-helices and seven β-sheets (fig. 4.4 B).

From aa440 the amino acid sequence passes the plasma membrane again to the cytosolic carboxy terminus. The plasma membrane distal and proximal parts are connected by two α-helices building a kind of "neck" region. This region is stiff and not bendable which might be due to interactions of the highly conserved residues Trp186, Leu199 and Leu323 (fig. 4.4 C).

The size of the AHK1-ED is ~10nm with a maximum diameter of ~5nm. The 63 amino acid residues which are identical in the similar sequences of AHK1-ED (fig. 4.3) are distributed all over the structure (fig. 4.4 A) indicating a high importance for the signal perception. According to the putative structure a mechanical perception of osmotic stress by AHK1 is unlikely. The identification of the PAS domain as well as the putative structure supports the idea of a low-molecular ligand binding to AHK1.

#### 4.1.2.3 Expression of AHK1-ED in *Escherichia coli* for CD-spectroscopy, crystallization and ligand fishing

The nucleotide sequence of *AHK1-ED* encoding aa99-446 of the AHK1 full length protein has been codon-optimized for expression in *Escherichia coli* by the company GenScript (USA) resulting in the vector construct *pUC57-AHK1-ED*. In this vector the nucleotide sequence of *AHK1-ED* was extended with a 5'-ATG and a 3'-double stop codon as well as recognition sites for the restriction endonuclease NotI at the very 5'-end and NcoI at the 3'-end which were subsequently used for the cloning of the codon-optimized sequence into the *E. coli* expression vector. The vector *pMH-HSsumo-AHK1-ED* for the expression of *AHK1-ED* under the control of a *T7-promoter* in *E. coli* was generated by classical cloning, fusing a His-tag, StrepII-tag and a small ubiquitin-related modifier (SUMO) to the amino terminus of AHK1-ED how it was described for AHK4 by Hothorn *et al.* (2011). In addition to AHK1-ED, the conserved Leu298 and Leu422 in the "neck"-region of AHK1-ED were mutated by site-directed mutagenesis on *pUC57-AHK1-ED* to Ala leading to *pUC57-AHK1-ED-Leu298/422Ala* and *pMH-Ssumo-AHK1-ED-Leu298/422Ala*. These mutations might influence the structure of AHK1-ED and might lead to an extracellular domain which is incapable of signal perception. The expression of the AHK1-ED fusion protein worked in Origami-2 (DE3) cells (appendix A19). With the use of the expression vectors *pMH-Ssumo-AHK1-ED* and *pMH-Ssumo-AHK1-ED-Leu298/422Ala*, the AHK1-ED and AHK1-ED-Leu298/422Ala fusion protein was expressed by GeneCust (L) with a purity of 80% (appendix A20). These proteins were then used for Circular Dichroism (CD)-spectroscopy in cooperation with Thilo Stehle and Volker Niemann to verify the secondary structure of the homology model of Michael Hothorn. Unfortunately the CD-spectroscopy did not work due to the high absorbance of sodium lauroyl sarcosine in the protein storage buffer. A change of buffer led to the almost complete loss of protein. The expression vector for the AHK1-ED fusion protein was further used for expression and native protein extraction by Thomas Drechsler to obtain protein for crystallization and ligand fishing as well as for antibody production (Drechsler, 2015).

### 4.1.3 AHK1-dependent signal transduction and adaption of the plants

When a certain signal is perceived by a receptor, signal transduction takes place, leads to altered gene expression and finally to an adaption of the plant to the altered surrounding. In previous studies *ahk1* knock down alleles in *Arabidopsis thaliana* were shown to have certain phenotypes.

#### 4.1.3.1 Stomata

Kumar *et al.* (2013) showed that the *ahk1* knock down lines *ahk1-1*, *ahk1-5* as well as *ahk1-6* had an increased abaxial stomatal index in comparison to the respective wildtype in 5-6 week old plants. In this study, this could also be shown for the cotyledons of three day old seedlings of *ahk1* knock down alleles in the Ws-2 ecotype whereas *ahk1-3/35S::AHK1-GFP* showed a higher significance of increased stomatal index in comparison to the wildtype than the knock down lines (fig. 4.5). Thereby, in this study seedlings were used as it was tried to investigate stomatal development under normal and osmotic stress conditions as it was described in Kumari *et al.* (2014). Unfortunately, stomatal density and stomatal index of mannitol stressed plants could not be determined as too many cells in each sample were damaged due to mannitol stress for which reason no proper outline of the cells could be imaged with the use of staining with neither propidium iodide nor FM4-64 and confokal microscopy.

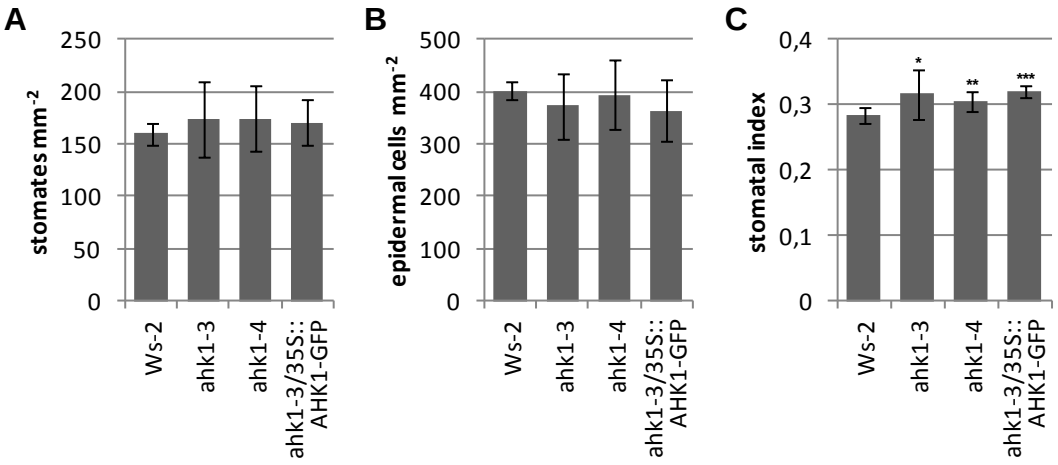



figure 4.5: Number of stomates and epidermal cells in *ahk1* knock down lines
Abaxial stomatal density (A), epidermal cell density (B) and stomatal index (C) of cotyledons of three day old seedlings. The cell walls of the cells have been stained with propidium iodide, images were taken using confokal microscopy (SP2) and used for the counting of cells. Data were obtained from one experiment with six cotyledons per line. Shown are mean values and standard deviations. Stars above bars show statistical significance of difference to the wildtype. Significance was calculated using student‘s ttest. *p<0.05; **p<0.01; ***p<0.0001

#### 4.1.3.2 Root growth of *ahk1* plants

Wohlbach *et al.* (2008) and Kumar *et al.* (2013) who were working with *ahk1* knock down alleles in different ecotypes obtained contradictory results in regard to root growth during osmotic stress conditions indicating a positive or rather negative effect of AHK1 on osmoregulation whereas they used different methods and growth conditions. Therefore the root growth of the *ahk1* knock down alleles was further investigated.

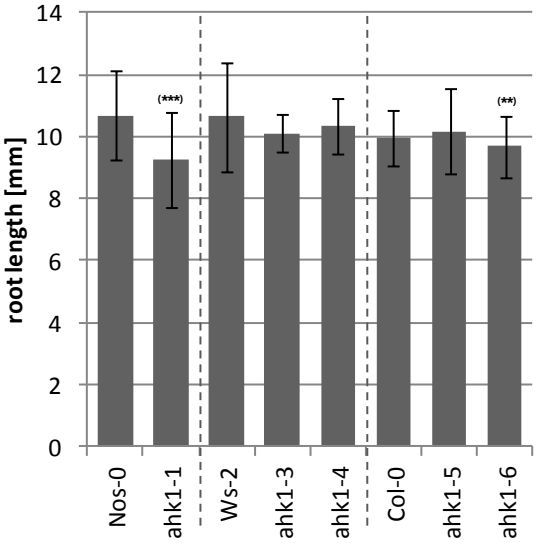



figure 4.6: Total root length of non-stressed *ahk1* knock down lines
Shown is the total root length in mm of four day old seedlings of *ahk1* knock down alleles in the three ecotypes Nos-0, Ws-2 and Col-0. The ecotypes are separated by dashed lines. The seedlings grew on half strength MS salts with 2mM MES and 1% phytoagar at constant light conditions and the root length has been measured with the use of ImageJ. Shown are the mean values and standard deviations of at least 21 seedlings per line and experiment. For the Nos-0 and Col-0 ecotype one experiment was executed, for the Ws-2 ecotype data of seven replicates were averaged. Student's t-test was used to calculate statistical significance. The stars above the bars display the significance: **p<0.01; ***p<0.001. Brackets around stars display that the significance has been shown in a single experiment.

First, the total root length of four day old seedlings of *ahk1* knock down alleles in the three different ecotypes Nos-0, Ws-2 and Col-0 was analyzed (fig. 4.6). In a single experiment with at least 21 seedlings per line *ahk1-1* in the Nos-0 ecotype and *ahk1-6* in the Col-0 ecotype showed significantly shorter roots than their wildtypes whereas *ahk1-5* did not show this difference. In averaged data of seven replicates with at least 21 seedlings per line *ahk1-3* and *ahk1-4* in the Ws-2 ecotype did not show a difference in root length in comparison to their wildtype. The total root length of the different ecotypes was generally similar and did not show any significant difference.

Wohlbach *et al.* (2008) used sorbitol media for osmotic stress treatment and did not investigate the total root length but the mean percentage of root elongation which was calculated based on a non-stressed control root. Thereby, *ahk1-3* and *ahk1-4* showed a reduced percentage of root elongation in comparison to their wildtype. In this study, in one experiment with at least 20 seedlings per line and treatment the results of Wohlbach *et al.* (2008) could not be reproduced. After four days growth on sorbitol-supplemented media the root elongation generally decreases with increasing amounts of sorbitol. The *ahk1* knock down alleles *ahk1*-3 and *ahk1-4* did not show a difference in the mean percentage of root elongation based on a non-stressed control root in comparison to the wildtype Ws-2 (fig. 4.7 H). The same is true for *ahk1-6* and Col-0 whereas *ahk1-5* showed a reduced mean percentage of root elongation on 100mM and 400mM sorbitol (fig. 4.7 I). The mean percentage of root elongation based on a non-stressed control root of *ahk1-1* in comparison to the wildtype Nos-0 was increased on 200mM and decreased on 400mM sorbitol (fig. 4.7 G).

Kumar *et al.* (2013) used a different method to quantify root elongation upon osmotic stress. They did not analyze the total root length but the total root elongation of seedlings in eight days growth on osmotic stress media. In this study, the total root elongation was determined four days after the transfer and also showed a general decrease in root elongation upon increasing amounts of sorbitol. The *ahk1* knock down allele *ahk1-1* showed an increased root elongation on 0mM, 50mM, 100mM and 200mM but a decreased root elongation on 400mM sorbitol in comparison to its wildtype Nos-0 (fig. 4.7 D). In the Ws-2 ecotype the *ahk1-3* and *ahk1-4* showed an increased root elongation in comparison to

their wildtype on 0mM, 50mM, 100mM and 200mM sorbitol whereas no significant difference could be detected for the root elongation on 400mM sorbitol (fig. 4.7 E). In the Col-0 ecotype *ahk1-5* showed a decreased root elongation in comparison to the wildtype on 100mM and 400mM sorbitol. *ahk1-6* showed an increased root elongation on 0mM and 50mM as well as a decreased root elongation on 400mM sorbitol (fig. 4.7 I).

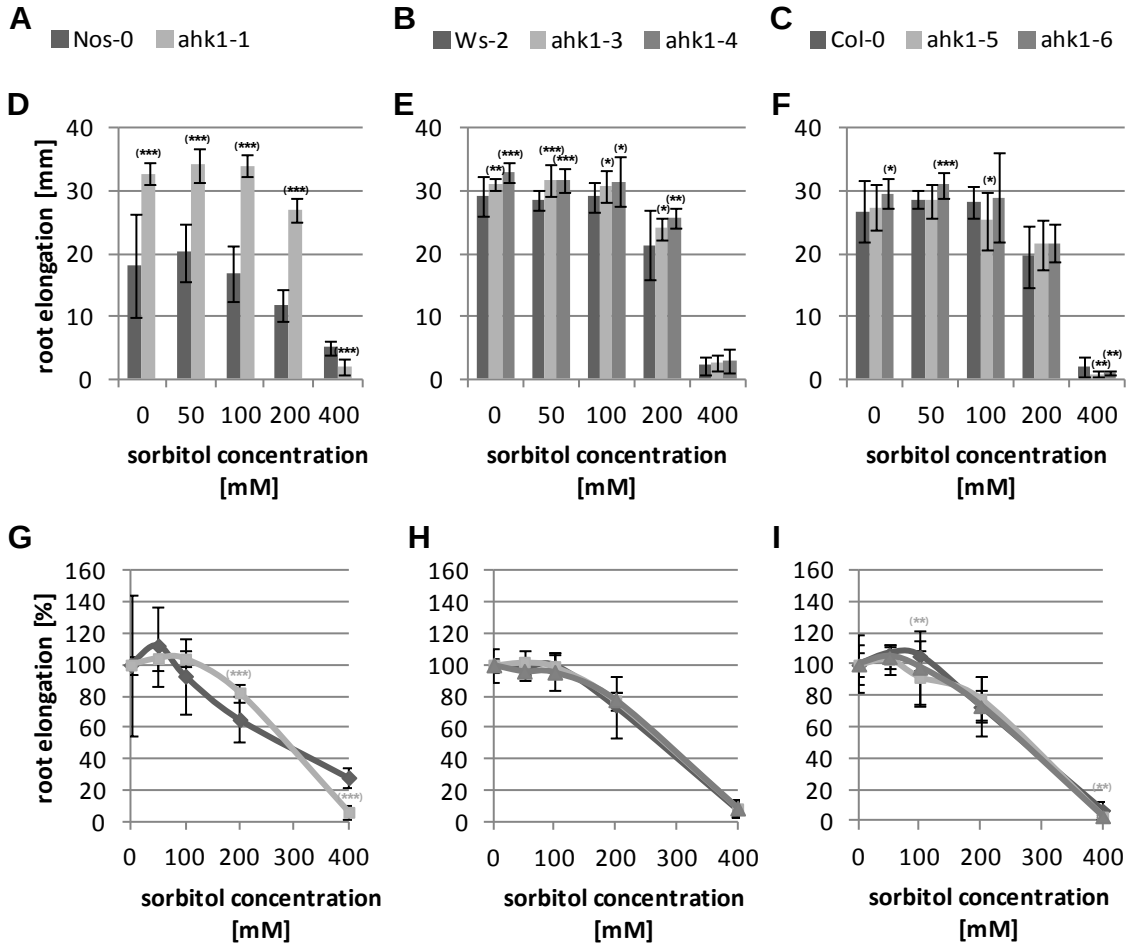



figure 4.7: Root elongation of *ahk1* knock down lines in different ecotypes during sorbitol stress.
The *ahk1* knock down alleles in the Nos-0 (A, D, G), Ws-2 (B, E, H) and Col-0 (C, F, I) ecotype were grown for four days on half strength MS salts at constant light conditions, were then transferred to sorbitol-supplemented media and grown for additional four days. The root elongation was analyzed with the method of Kumar *et al.* (2013) showing the total root elongation (D, E, F) and the method of Wohlbach *et al.* (2008) showing the mean percentage of root elongation based on a non-stressed control root (G, H, I). (A) gives the color code for (D) and (G), (B) gives the color code for (E) and (H), (C) gives the color code for (F) and (I). The labeling of the y-axis in (D) is also valid for the y-axes in (E) and (F) the labeling of the y-axis in (G) is also valid for the y-axes in (H) and (I). Shown are mean values and standard deviations of one experiment with at least 20 seedlings per line and treatment. Student‘s t-test was used to analyze statistical significance of differences. Stars above the bars display statistical significance in comparison to the respective wildtype. *p<0.05; **p<0.01; ***p<0.001. Brackets around stars display that the significance has been shown in just one experiment.

In summary the *ahk1* knock down alleles in the Nos-0 and Ws-2 ecotype showed a generally increased root elongation in comparison to their wildtypes on the control media as well as on osmotic stress media. This could not be shown in the Col-0 ecotype. In contrast, the root elongation of the *ahk1* knock

down alleles in the Nos-0 and Col-0 ecotype on 400mM sorbitol is decreased in comparison to their wildtype which could not be shown in the Ws-2 ecotype. This indicates that osmotic stress or at least sorbitol is not causing the difference in root elongation. Furthermore, the method which is used for the analysis of root growth during osmotic stress gives different results for the same raw data.

Another possibility to apply osmotic stress is the use of mannitol. Mannitol has also been used in previous studies about AHK1 by Katharina Caesar. Therefore it was tested whether the root elongation of the *ahk1* knock down alleles behaves similar during mannitol stress like during sorbitol stress at the same growth conditions. It could be revealed that this is the case.

In one experiment with at least 20 seedlings per line and treatment a general decrease in root elongation could be correlated to increasing amounts of mannitol like previously shown with sorbitol. A decrease in the mean percentage of root elongation based on a non-stressed control root in comparison to the respective wildtype could be detected for *ahk1-1* grown on 50mM mannitol (appendix A21 G), for *ahk1-3* grown on 200mM and 400mM mannitol (appendix A21 H), for *ahk1-4* grown on 50mM, 200mM and 400mM mannitol (appendix A21 H), for *ahk1-5* grown on 400mM mannitol (appendix A21 I) and for *ahk1-6* grown on100mM and 200mM mannitol (appendix A21 I). An increase could be shown exclusively for *ahk1-5* grown on 100mM mannitol (appendix A21 I).

In total root elongation *ahk1-1* showed a general increase in comparison to its wildtype (appendix A21 D). In the Ws-2 ecotype *ahk1-3* showed an increased root elongation in comparison to the wildtype on 0mM and 100mM mannitol, whereas *ahk1-4* showed an increased root elongation on 0mM and a decreased root elongation on 400mM mannitol (appendix A21 E). In the Col-0 ecotype an increased root elongation was detected for *ahk1-5* grown on 100mM mannitol and for *ahk1-6* grown on 0mM and 50mM mannitol. On 400mM mannitol the root elongation of *ahk1-5* and *ahk1-6* was decreased in comparison to the wildtype (appendix A21 F).

The root elongation of *ahk1* knock down alleles on mannitol-supplemented media is as contradictory as the root elongation on sorbitol.

So far the root elongation was analyzed in eight day old seedlings which have been grown on osmotic stress media for four days. Wohlbach *et al.* (2008) analyzed the mean percentage of root elongation in eight day old seedlings after five days growth on osmotic stress media whereas Kumar *et al*. (2013) analyzed the root elongation in twelve day old seedlings after eight days of growth on stress media. Hence, the root elongation on mannitol-supplemented media of *ahk1-3*, *ahk1-4* and *ahk1-3/35S::AHK1-GFP* in comparison to the wildtype Ws-2 was analyzed four and eight days after the transfer of four day old seedlings and compared.

Averaged data of three experiments with at least 19 seedlings per *Arabidopsis thaliana* line and treatment did not show any significant difference in total root elongation (fig. 4.8 B, C) or in the mean percentage of root elongation based on a non-stressed control root (fig. 4.8 D,E) after neither four (fig. 4.8 B, D) nor eight days (fig. 4.8 C, E) of osmotic stress treatment. Like in previous experiments a general decrease in root elongation upon increasing osmotic stress could be observed.

The osmotic stress dependent difference in root elongation of *ahk1* knock down alleles how it was described by Wohlbach *et al.* (2008) and Kumar *et al.* (2013) could not be shown in additional replicates of the mannitol stress experiment.

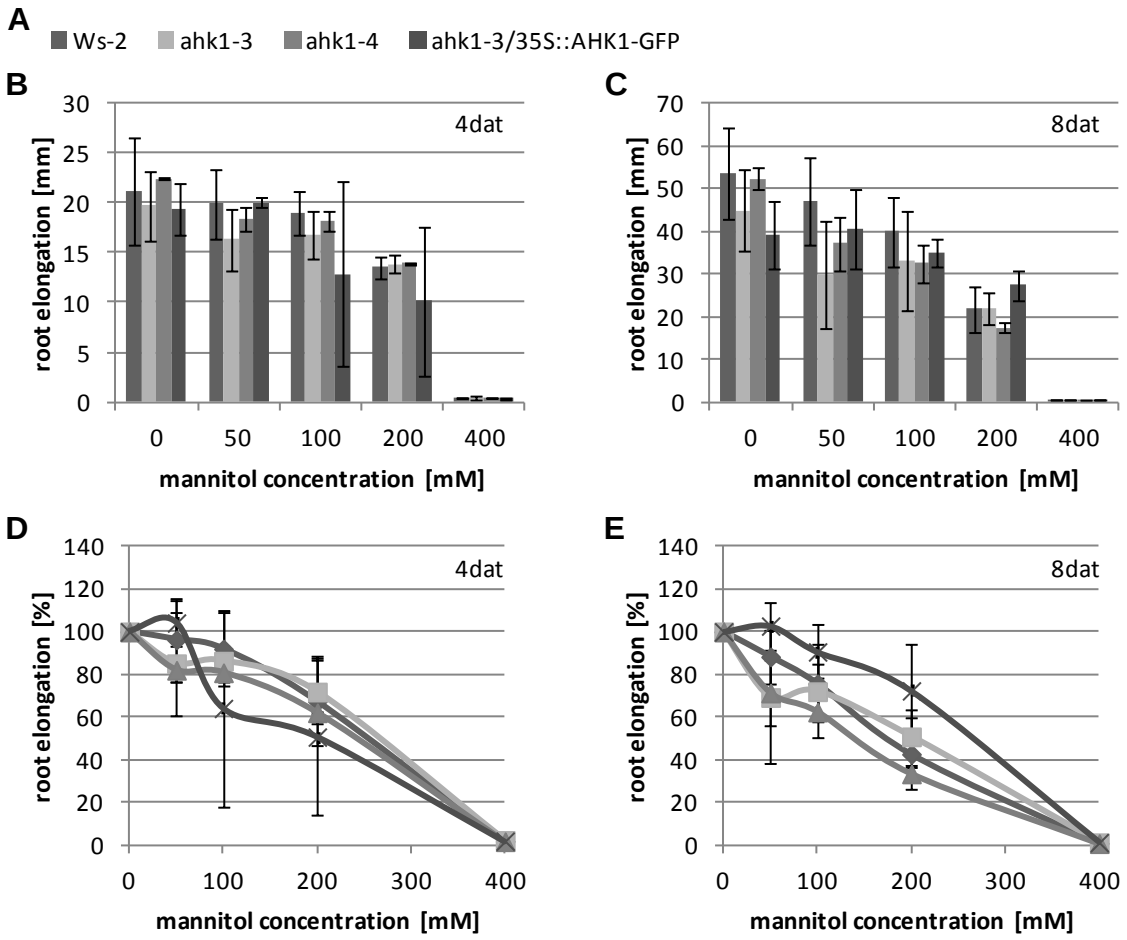



figure 4.8: Comparison of the root elongation of *ahk1* mutants in four and eight days of mannitol stress Ws-2 (dark grey), *ahk1-3* (light grey), *ahk1-4* (medium grey) and ahk1-3/*35S::AHK1-GFP* (darkest grey) were grown on half strength MS salts without mannitol for four days at constant light conditions and then transferred to mannitol-supplemented media. Four days (4dat; B, D) and eight days (8dat; C, E) after the transfer the root elongation was measured. Neither the analysis with the method of Kumar *et al.* (2013) (B, C) showing the total root elongation nor the method of Wohlbach *et al.* (2008) (D, E) showing the mean percentage of root elongation based on a non-stressed control root could show a significant difference of the root elongation of *ahk1* mutants in the Ws-2 ecotype after four (B, D) and eight days (C, E) of mannitol treatment. Shown are mean values and standard deviations of three experiments with at least 19 seedlings per line and treatment. (A) gives the color code for (B), (C), (D) and (E).

Dependent on the season when the root growth was investigated contrary results were obtained (fig. 4.9). In three experiments (exp.1-3) which were executed during winter time the root length (fig. 4.9 B) and elongation (fig. 4.9 D) of *ahk1-3* and *ahk1-4* was increased in comparison to the wildtype whereas in three experiments in summer time (exp.4-6) the total root length after (fig. 4.9 B) and the root elongation (fig. 4.9 D) in four days of growth on osmotic stress of *ahk1-3* and *ahk1-4* was not changed in comparison to the wildtype whereby the plants were grown in the same phytochamber with same settings and experimental setup. Still, in summer time the phytochamber had problems to reduce the temperature to the set value. For a better comparison of the root length and elongation between the different experiments the root length and elongation of the *ahk1* knock down lines was normalized to the respective wildtype root which grew at the same conditions. The averaged data of all replicates do

not show a difference in root length (fig. 4.9 C) and root elongation (fig. 4.9 E) between *ahk1* knock down alleles and their wildtype upon osmotic stress treatment.

In summary the root growth phenotypes for the *ahk1* knock down alleles were contradictory in the respective publications and could not be reproduced in this study. This arose the question about the components of the AHK1 signal transduction pathway and a reproducible AHK1-dependent phenotype.

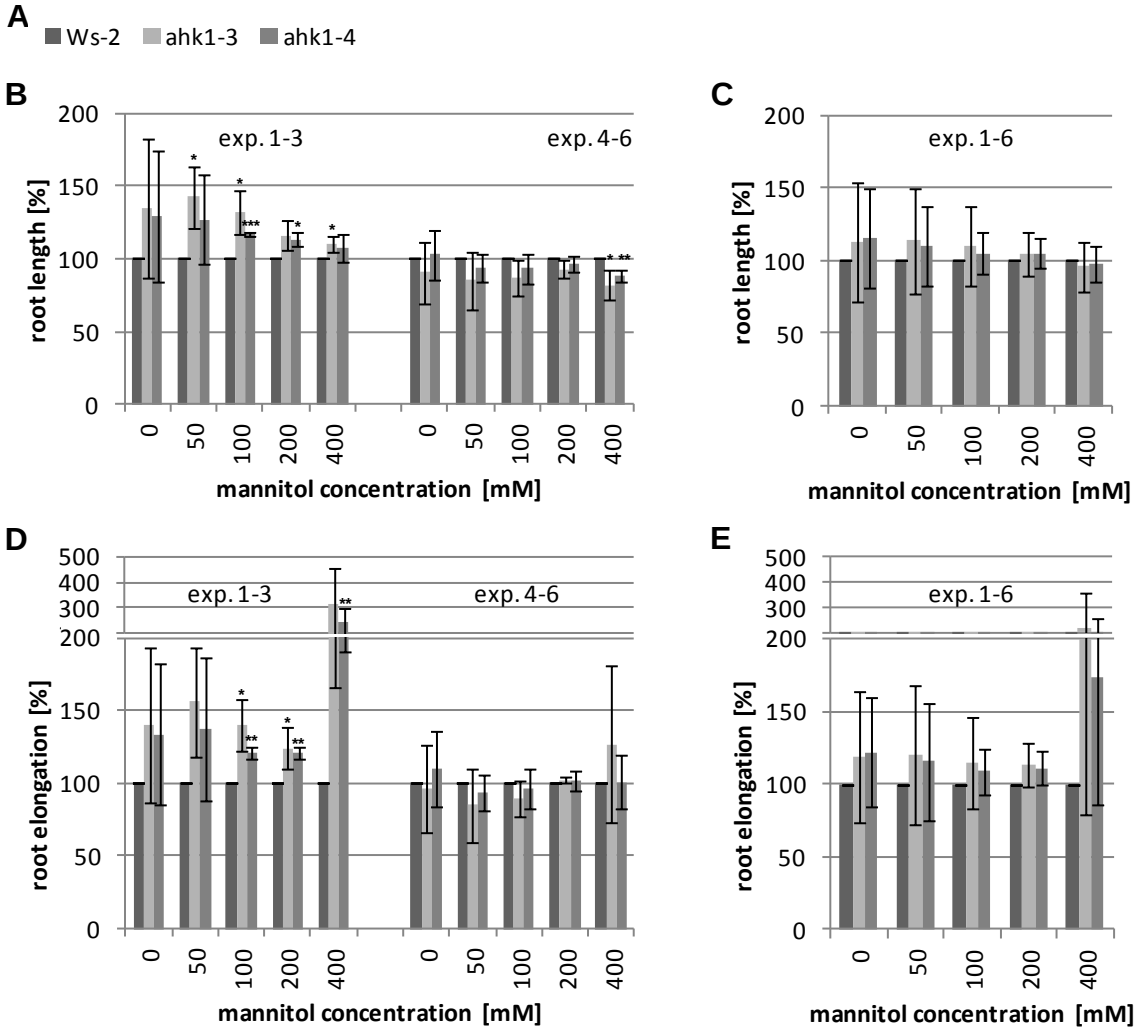



figure 4.9: Root growth of *ahk1* knock down lines is not stably reproducible during mannitol stress.
(A) gives the color code for (B) to (D). Eight day old seedlings of *ahk1* knock down lines which have been grown on mannitol-supplemented media for four days do not show a stably reproducible phenotype during mannitol stress. Shown is the mean percentage of the total root length (B, C) and the mean percentage of root elongation (D, E) with the respective standard deviation of six experiments with at least four (exp. 1-3) or 19 (exp. 4-6) eight day old seedlings per line and treatment. Experiment 1-3 (exp.1-3) have been conducted during winter time, experiment 4-6 (exp. 4-6) during summer time. (B) and (D) show the season dependence of the results, (C) and (E) show the mean values of all experiments. The mean value of the root length as well as the root elongation of the respective wildtype was defined as 100%. Student's t-test was used to calculate statistical significance within the defined experiments. Stars above the bars indicate the statistical significance of difference to the respectively treated wildtype: *p<0.05; **p<0.01; ***p<0.001

#### 4.1.3.3 Phosphoproteome of *ahk1-3* and its wildtype Ws-2

AHK1 is part of the multistep phosphorelay system in plants in which the signal is usually transduced by the transfer of one phosphate from histidine (His) to aspartate (Asp) residues which finally changes the activity of response regulators and subsequently regulates gene expression. The phosphorylation of His and Asp is biochemically not as stable as serine (Ser)/threonine (Thr) or tyrosine (Tyr) phosphorylation and a direct transfer of the phosphate from His or Asp to Ser/Thr or Tyr is not possible (Sanders *et al.*, 1989) but as it is known from the osmotic stress signaling HOG-pathway in yeast (Reiser *et al.*, 2003) as well as from the ethylene signaling pathway in plants (Merchante *et al.*, 2013) a transition from the multistep phosphorelay system to a classical phosphorylation cascade can occur. To analyze whether such a transition also occurs AHK1-dependent upon osmotic stress in *Arabidopsis thaliana*, the phosphoproteome of *ahk1-3* was compared to the wildtype (wt) Ws-2 after 10min of 0.3M mannitol or mock treatment in a reciprocal metabolic labeling experimental design as well as in a non labeling experimental design.

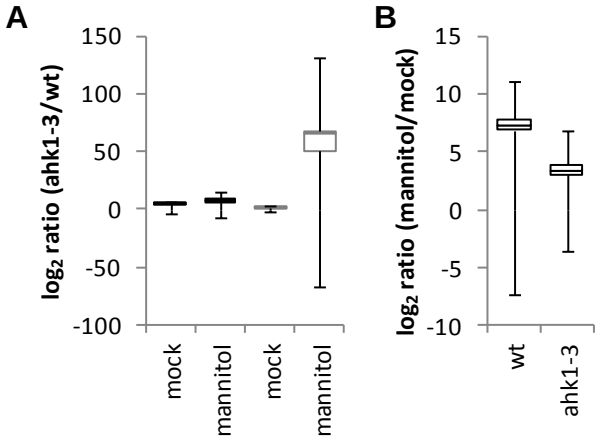



figure 4.10: Distribution of phosphopeptide $log_2$ ratios
(A) Distribution of phosphopeptide $log_2$ ratios between the *ahk1-3* mutant and the wildtype in the mock and mannitol treated experiments with the experimental setup of no labeling (black) and metabolic labeling (grey). (B) Distribution of phosphopeptide $log_2$ ratios between mannitol and mock treatment in the wildtype and the *ahk1-3* mutant in the experimental setup without labeling.

In these experiments, which have been executed by Waltraud X. Schulze, peptides with specific phosphorylation patterns (phosphopeptides) at Ser, Thr or Tyr residues were identified and quantified by mass spectrometry. The $log_2$-value of the ratio of normalized phosphopeptide ion intensities of *ahk1-3* and the wildtype Ws-2 simplifies the analysis whether a phosphopeptide is more abundant in *ahk1-3* ($log_2>0$) or in the wildtype ($log_2<0$). The altered abundance of phosphopeptides is further termed differentially phosphorylated whereas a $log_2$-value above 1.0 or beyond -1.0 which indicates a two times higher or respectively lower abundance of the phosphopeptide in *ahk1-3* in comparison to the wildtype was chosen as threshold. This threshold was chosen as due to insufficient data points for the ttest it was not possible to calculate the significance of difference for each phosphopeptide and because for the experiment without labeling it has to be taken into account that proteins from the different samples were extracted, further processed and analyzed independently for each line and treatment which can lead to quantitation errors. In figure 4.10 the distribution of $log_2$ ratios for all experiments is illustrated. It reveals a differential phosphorylation between *ahk1-3* and the wildtype upon mannitol and mock treatment indicating an AHK1-dependent transition from the multistep phosphorelay system to classical phosphorylation cascades. Additionally a general and mannitol-dependent differential phosphorylation could be observed in *ahk1-3* as well as in the wildtype. A high

range of $log_2$-values could be shown especially for phosphopeptides which were quantified in the metabolic labeling experiment with mannitol treatment.

In these experiments in total 3022 phosphopeptides were quantified (fig. 4.11) whereas 1055 phosphopeptides were quantified in the reciprocal metabolic labeling experiment with *ahk1-3* and the wildtype after mannitol treatment (appendix A33). In the reciprocal metabolic labeling experiment with *ahk1-3* and the wildtype just 140 phosphopeptides could be quantified after mock treatment (appendix A34). In the experiment without labeling but after mannitol and mock treatment of *ahk1-3* and the wildtype 1827 phosphopeptides were quantified (appendix A35).

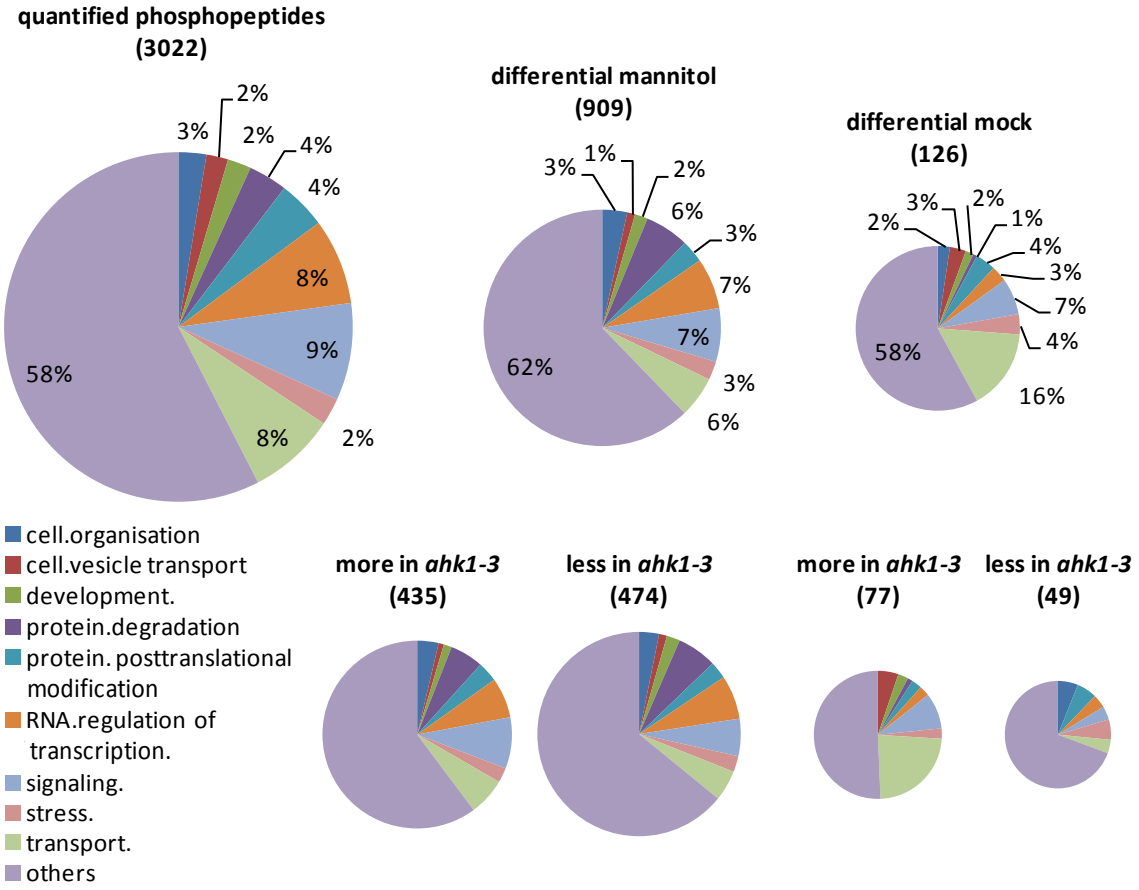



figure 4.11: Functional categorization of the quantified phosphopeptides.

Functional categorization of phosphopeptides which were quantified in experiments with metabolic labeling and without labeling. In total 3022 phosphopeptides were quantified and categorized according to their function to the categories cell.organisation (dark blue), cell.vesicle transport (red), development (dark green), protein.degradation (dark violet), protein.posttranslational modification (petrol), RNA, regulation of transcription (orange), signaling (light blue), stress (rosé), transport (light green) and others (light violet). The category “others” comprises cell wall, cell, DNA, gluconeogenesis/glyoxylate cycle, glycolysis, metal handling, microRNA, misc, protein, photosynthesis, redox, RNA, TCA/org transformation, not assigned, secondary metabolism and metabolism of amino acids, carbohydrates, nucleotides, nitrate, hormones, lipids, Co-factors and vitamins. Differential mannitol and differential mock describes phosphopeptides which show a $log_2$-value above 1.0 or beyond -1.0. The phosphopeptides of “differential mannitol (909)” were subgrouped into “more in *ahk1-3* (435)” and “less in *ahk1-3* (474)” whereas the phosphopeptides of “differential mock” were subgrouped into “more in *ahk1-3* (77)” and “less in *ahk1-3* (49)”. Same colors designate the same functional category.

After mannitol treatment in total 909 phosphopeptides were differentially phosphorylated in *ahk1-3* in comparison to wt whereas 435 peptides showed more and 474 showed less phosphorylation (fig. 4.11). After mock treatment, in total 126 phosphopeptides were differentially phosphorylated whereas 77 peptides showed more and 49 showed less phosphorylation (fig. 4.11). The portions of the functional categories to which the quantified phosphopeptides were sorted did not reveal blatant changes after mannitol but after mock treatment especially in the portions of phosphopeptides which are involved in “cell.vesicle transport”, “development.”, “RNA.regulation of transcription.” “signaling.”, “stress.” and “transport.”. All quantified phosphopeptides, their $log_2$-values as well as their functional categories are listed in appendix A33, A34 and A35 respectively.

#### 4.1.3.4 Components of the multistep phosphorelay system

Interestingly, some components of the multistep phosphorelay system were identified and quantified in the phosphoproteomic analysis of *ahk1*-3 and Ws-2 comprising Ser and Thr phosphorylation. The components and the respective quantified phosphopeptides are listed in table 4.1. After 10min treatment with mannitol AHK4 (gi:30677959) showed a highly reduced phosphorylation at Ser875 and Thr883 and ARR19 (gi:334183176) at Ser171 whereas the phosphorylation of AHK2 (gi:18421494) at Thr11 and Ser12 is similar in *ahk1-3* in comparison to the wildtype. EIN4 (gi:42572247) with phosphorylated Ser635 and Ser637 was identified in just one line and treatment so no comparative quantification could be obtained. As these phosphorylation sites are Ser and Thr residues which are located neither in the histidine kinase domain nor in the receiver domain (fig. 4.12) they do not directly belong to the multistep phosphorelay system which occurs between His and Asp residues. In the phosphoproteomic analysis of *ahk2 ahk3* in comparison to the wildtype Col-0 AHK2 was also found to be phosphorylated at Ser and Thr residues but at Thr4, Ser596 and Thr740. Most of these phosphorylation sites are identified to be close to NEK2-kinase sites (Dinkel *et al.*, 2016). There are seven NEK-kinases annotated in *Arabidopsis thaliana* but no phosphopeptide of them was quantified in the phosphoproteome of *ahk1-3* and wt.

table 4.1: Quantified phosphopeptides of components of the multistep phosphorelay system.
$log_2$-values for the ratio of quantified peptides in *ahk1-3* and the wildtype (wt) Ws-2 in the experiments with a reciprocal metabolic labeling (met. labeling) experimental design or without labeling (no labeling) after 10min treatment with 0.3M mannitol (+man) or mock respectively (-man). For met. labeling +man $log_2$-values of the two reciprocal replicates are shown. The phosphorylated residue is highlighted with a subsequent (ph). (ac) reveals acetylation, (ox) oxidation. The “x” shows that the $log_2$-value could not be calculated due to a not quantified peptide for the nominator or denominator. “-“ marks no quantification. Underlined values revealed statistical significance with $p<0.05$. Data obtained from Waltraud X. Schulze.

| accession | name | phosphopeptide | $log_2$-values (*ahk1-3*/wt) | | | | |
|---|---|---|---|---|---|---|---|
| | | | met. labeling | | | no labeling | |
| | | | +man | | -man | +man | -man |
| AT5G35750 | AHK2 | (ac)SITCELLNLT(ph)S(ph)KK | - | -0.09 | - | - | - |
| AT2G01830 | AHK4 | TNGNVHHKS(ph)PKLALFAT(ph)NITNSEFDR | -4.55 | -8.99 | - | - | - |
| AT3G04580 | EIN4 | SILAGNAPELQHPNS(ph)NSILR | - | - | - | x | x |
| | | SILAGNAPELQHPNSNS(ph)ILR | - | - | - | <u>x</u> | x |
| AT1G49190 | ARR19 | S(ph)DRLDQVK | -20.44 | - | - | - | - |

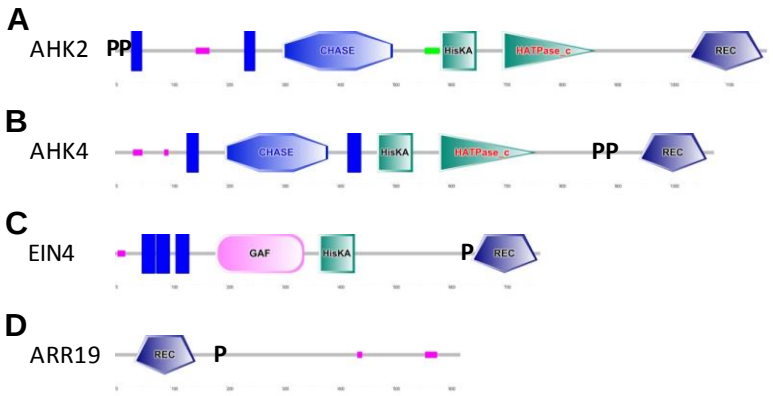



figure 4.12: Ser/Thr-phosphorylation sites in components of the multistep phosphorelay system
Schematic drawings of the domain structure of AHK2 (A), AHK4 (B), EIN4 (C) and ARR19 (D) according to the SMART database. Phosphorylation sites identified in this study are indicated by "**P**". The blue octagon indicates the CHASE domain, the green rectangle the histidine kinase domain (HisKA), the green triangle the HATPase_c domain, the pentagon the receiver domain, blue bars the transmembrane domains, magenta bars regions of low complexity and green bars coiled-coil regions.

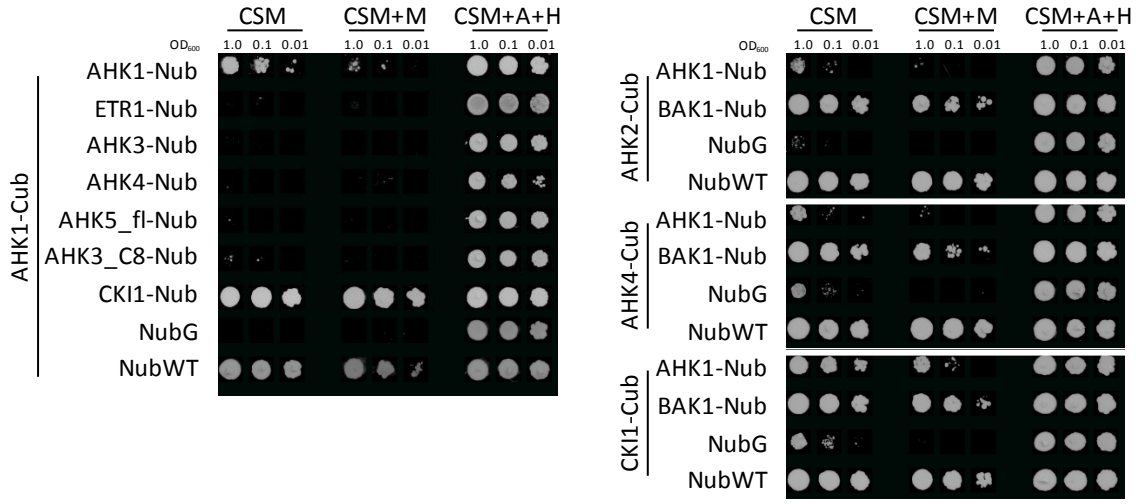



figure 4.13: Mating-based split-ubiquitin assay with components of the multistep phosphorelay system
Fusion constructs of *AHK1*, *AHK2*, *AHK4* and *CKI1* with the C-terminal part of *ubiquitin* (*Cub*) were transformed into the *S. cerevisiae* strain THY.AP4, fusion constructs of *AHK1*, *ETR1*, *AHK3*, *AHK4*, *AHK5* in full length (*AHK5_fl*) and as truncated version (*AHK1-C8*), *CKI1* and *BAK1* with the N-terminal part of *ubiquitin* (*Nub*) as well as the negative control *NubG* and the positive control *NubWT* were transformed into the *S. cerevisiae* strain THY. AP5. After the mating the interaction of the proteins was tested by dropping of the yeast in the concentration of $OD_{600}$=1.0, $OD_{600}$=0.1 and $OD_{600}$=0.01 on CSM minimal medium (CSM) and verified by dropping them on CSM minimal medium supplemented with 50µM Met (CSM+M) whereas CSM-Ade$^+$-His$^+$ (CSM+A+H) served as growth control. The growth was recorded after four days growth at 28°C. The detection of protein expression is shown in appendix A22.

AHK1 is known to form homodimers (Caesar, unpublished). With the identified AHK1-dependent Ser and Thr phosphorylation of AHK2 and AHK4 the question arose, whether AHK1 also forms heterodimers with other AHKs although a direct phosphorylation of AHK2 and AHK4 from His or Asp to Ser or Thr is biochemically not possible (Sanders *et al.*, 1989). With the use of the mating-based split-ubiquitin (mbSUS) system in *Saccharomyces cerevisiae* it could be shown, that AHK1 does not

interact with ETR1, AHK3 and AHK5 in full length (AHK5_fl) or in a truncated version (AHK5_C8) but interacts weakly with AHK2 and AHK4 and strongly with CKI1 (fig. 4.13).

In contrast to AHK2, AHK3 and AHK4 which localize to the endoplasmic reticulum (Caesar *et al.*, 2011b) CKI1-RFP co-localizes with AHK1-GFP in transient expression in *N. benthamiana* leaves at the plasma membrane as well as in vesicle-like structures which were described in 4.1.1.2 (fig. 4.14). Unfortunately the expression of AHK1 and CKI1 was too low for FRET-FLIM measurements. To investigate, whether the interaction of AHK1 and CKI1 reveals a physiological function mesophyll protoplasts of Ws-2, *ahk1-3* and *ahk1-4* were transfected with a construct encoding a *luciferase* (*LUC*) under the control of the *ARR5-promoter* (*pBT8-ARR5::LUCm3*) which is CKI1- and kinetin-dependently induced (Hejatko *et al.*, 2009). Unfortunately no results could be obtained as the protoplasts were not stable enough for the time of the protocol. This was also true for these protoplasts which were transfected with a construct encoding a *LUC* under the control of the *RD29B-promoter* (*pB7-RD29Bpro-LUCm-3XHA*) and treated with different concentrations of mannitol as well as for these protoplasts which were transfected with a construct encoding a *LUC* under the control of the *pFRK1-promoter* (*pFRK::LUC nos c*) and treated with a mixture of the PAMPs elf18 and flg22.

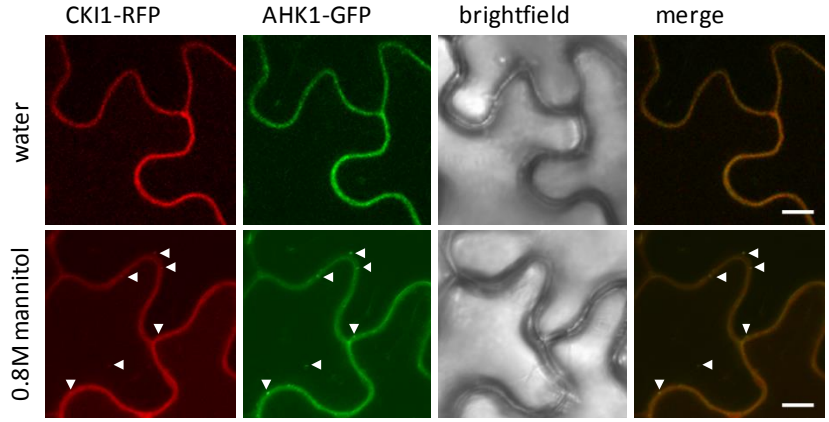



figure 4.14: Localization of CKI1-RFP and AHK1-GFP
Transient co-expression of *CKI1* tagged with a C-terminal *RFP* (*CKI1-RFP*, red) and *AHK1* tagged with a C-terminal *GFP* (*AHK1-GFP*, green) under the control of the *CaMV 35S-promoter* in *N. benthamiana* revealed in confokal microscopy that CKI1-RFP and AHK1-GFP co-localize at the plasma membrane and after treatment with 0.8M mannitol additionally in vesicle-like structures (white arrows). The scale is 10µm.

#### 4.1.3.5 Mitogen-activated protein kinases

In the phosphoproteome of *ahk1-3* and the wildtype Ws-2 several kinases were quantified (appendix A24) including some MAP kinases (table 4.2).

In a yeast two-hybrid assay the intracellular part (ICP) of AHK1 comprising amino acid 470-1207 was tested for interaction with different MAP kinases. AHK1-ICP did not show any interaction with MKKK20, MPK2, MPK3, MPK4, MPK5, MPK6, MPK7, MPK11 or MPK17 (appendix A26). Furthermore, in a mbSUS assay the interaction of AHK1 and MKKK20 was tested and neglected (appendix A25). So far it has not been tested whether ARRs downstream of AHK1 but especially ARR19 which was quantified in the phosphoproteome and identified to be phosphorylated at Ser171 interact with any of the MAP kinases which were also identified in the phosphoproteome.

table 4.2: Quantified phosphorylated peptides of MAP kinases
$log_2$-values for the ratio of quantified peptides in *ahk1-3* and the wildtype (wt) Ws-2 in the experiments with a reciprocal metabolic labeling (met. labeling) experimental design or without labeling (no labeling) after 10min treatment with 0.3M mannitol (+man) or mock respectively (-man). For met. labeling +man $log_2$-values of the two reciprocal replicates are shown.The phosphorylated residue is highlighted with a subsequent (ph). (ac) reveals acetylation, (ox) oxidation. The "x" shows that the $log_2$-value could not be calculated due to a not quantified peptide for the nominator or denominator. "-" marks no quantification. Underlined values revealed statistical significance with $p<0.05$. Data obtained from Waltraud X. Schulze.

| accession | name | phosphopeptide | $log_2$-values (*ahk1-3*/wt) | | | | |
|---|---|---|---|---|---|---|---|
| | | | met. labeling | | | no labeling | |
| | | | +man | | -man | +man | -man |
| AT1G53165 | ATMAP4K ALPHA1 | EFS(ph)SNANFSPLAR | - | - | - | x | -0.69 |
| AT3G58640 | MAPKKK-related | AISLPSS(ph)PQNYR | - | - | - | x | <u>-0.65</u> |
| | | SIS(ph)ITPEIGDDIVR | - | - | - | 0.07 | <u>-0.71</u> |
| AT3G13530 | MAPKKK7 | TPS(ph)SVSGNELAR | - | - | - | x | 0.82 |
| | | VRS(ph)GQLDPNNPIFGQNETSSLSM(ox)IDQPDVLK | - | - | - | x | x |
| AT4G08500 | MAPKKK8 | S(ph)LEFPEPTSFR | - | - | - | x | <u>x</u> |
| AT1G07150 | MAPKKK13 | M(ox)LS(ph)S(ph)PSSFWVR | - | -1.21 | - | - | - |
| AT3G50310 | MAPKKK20 | DEDKVLMS(ph)PK | - | 2.84 | - | - | - |
| AT4G29810 | MKK2 | FLTQSGT(ph)FK | - | - | - | x | x |
| | | IISQLEPEVLS(ph)PIKPADDQLSLSDLDM(ox)VK | - | - | - | <u>x</u> | x |
| AT1G53510 | MPK18 | FS(ph)KADPLALR | - | - | - | - | - |

#### 4.1.3.6 Link of AHK1 to the cytoskeleton

The phosphoproteome of *ahk1-3* and Ws-2 revealed several differentially phosphorylated peptides which are involved in cell organization (fig. 4.11, appendix A33, A34, A35). Some of these quantified peptides were tested in a yeast two-hybrid assay for interaction with AHK1-ICP. This interaction study was executed in the bachelor thesis of Achim Lorenz (2014). The empty vector controls were placed here in appendix A26 although the detection of the proteins in Western Blots has to be repeated.

AHK1-ICP shows interaction with ATPase katanin p60 (AT1G80350) and a putative myosin (AT5G20470) (appendix A26). Therefore it was interesting whether the assembly, disassembly and general arrangement of the cytoskeleton is altered in the *ahk1* knock down alleles. To address this question the binary plant vector constructs *pUBN-RFP-MBD* and *pUB-GFP-ABD2-GFP* for the expression of a RFP-tagged microtubule-binding protein (MBP) and a GFP-tagged actin-binding protein were separately stably transformed into all *ahk1* knock down alleles and the respective wildtypes. This did not work in the Ws-2 ecotype. In the Col-0 ecotype the lines were progenated and heterozygous T2-lines could be obtained and now be used for the further analysis of the cytoskeleton as homozygous plants reveal severe developmental defects.

#### 4.1.3.7 Effect of AHK1 on proteins involved in the response to light

The analysis of the phosphoproteome of *ahk1-3* and Ws-2 and the functional categories of the quantified phosphopeptides put proteins into focus which contribute to the plant's response to light (table 4.3).

SPA2, COP1, HY5 and FHY3 are involved in the regulation of photomorphogenesis of seedlings (Jaedicke *et al.*, 2012; Chen *et al.*, 2015; Siddiqui *et al.*, 2016). Therefore it was tested whether *ahk1*

knock down alleles show differences in comparison to their wildtype in the length of their hypocotyl or root after they were grown for three days in the dark.

table 4.3: Quantified phosphopeptides of proteins involved in the response to light
$log_2$-values for the ratio of quantified peptides in *ahk1-3* and the wildtype (wt) Ws-2 in the experiments with a reciprocal metabolic labeling (met. labeling) experimental design or without labeling (no labeling) after 10min treatment with 0.3M mannitol (+man) or mock respectively (-man). For met. labeling +man $log_2$-values of the two reciprocal replicates are shown.The phosphorylated residue is highlighted with a subsequent (ph). (ac) reveals acetylation, (ox) oxidation. The "x" shows that the $log_2$-value could not be calculated due to a not quantified peptide for the nominator or denominator. "-" marks no quantification. Underlined values revealed statistical significance with p<0.05. Data obtained from Waltraud X. Schulze.

| accession | name | phosphopeptide | $log_2$-values (*ahk1-3*/wt) | | | | |
|---|---|---|---|---|---|---|---|
| | | | met. labeling | | | no labeling | |
| | | | +man | | -man | +man | -man |
| AT4G11110 | SPA2 | ARNMDQQT(ph)VAS(ph)SGSALVIANTSAK | 0.50 | - | - | - | - |
| AT2G32950 | COP1 | M(ox)EEIS(ph)T(ph)DPVVPAVKPDPR | 4.18 | - | - | - | - |
| AT5G11260 | HY5 | EGIES(ph)DEEIR | - | - | - | 0.02 | 0.24 |
| AT3G22170 | FHY3 | SLPDVVTS(ph)PTQGLISVEEDNHSR | - | - | - | -0.94 | x |

In four replicates with at least 18 seedlings per line the etiolated *ahk1* knock down seedlings of the Nos-0 and Ws-2 ecotype indeed showed a reduced hypocotyl and root length in comparison to the respective wildtype. Thereby, the averaged hypocotyl and root length of the respective wildtype was defined as 100%. The *ahk1-3*/*35S::AHK1-GFP* line in the Ws-2 ecotype obtained no difference of hypocotyl or root length in comparison to the wildtype indicating a complementation of the decreased hypocotyl and root length in *ahk1-3* by the expression of AHK1-GFP. In the Col-0 ecotype *ahk1-5* did not show any difference in comparison to the wildtype whereas *ahk1-6* showed a reduced and the AHK1 overexpressor line revealed an increased hypocotyl length (fig. 4.15).

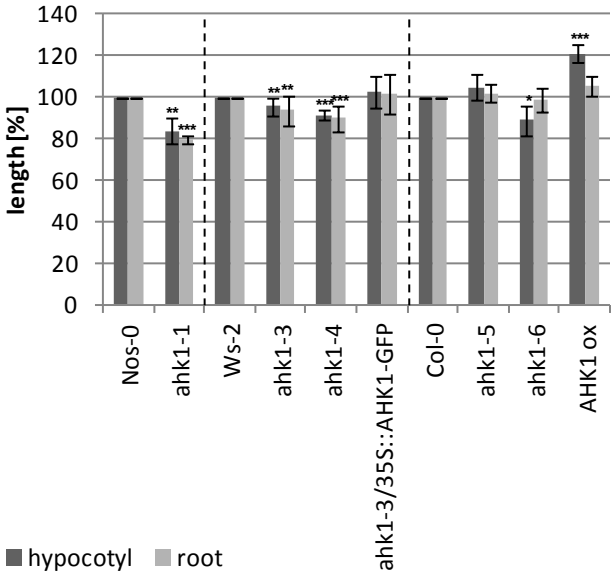



figure 4.15: Hypocotyl and root length of etiolated *ahk1* knock down seedlings
Hypocotyl (dark grey) and root (light grey) length of seedlings of *ahk1* knock down alleles in the Nos-0, Ws-2 and Col-0 ecotype which grew in the dark for three days after 2h light induction of germination. The hypocotyl and root length has been normalized to the respective wildtype. The ecotypes are separated by dashed lines. Data were averaged between at least four replicates with at least 18 seedlings per line and treatment. Shown are mean values and standard deviations. Student's ttest was used to calculate statistical significance. Stars above bars reveal statistical significance of difference in comparison to the wildtype. *p<0.05; **p<0.01; ***p<0.001

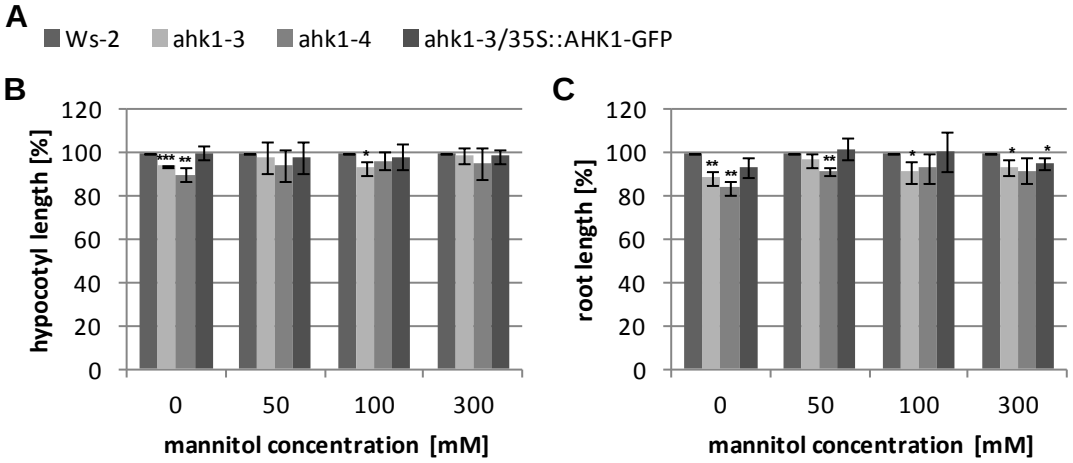



figure 4.16: Hypocotyl and root length of etiolated seedlings upon mannitol treatment
Hypocotyl (B) and root (C) length of seedlings of *ahk1-3*, *ahk1-4*, *ahk1-3/35S::AHK1-GFP* and their wildtype Ws-2 which grew on 0mM, 50mM, 100mM and 200mM mannitol in the dark for three days after 2h light induction of germination. The color code for the lines in (B) and (C) is given in (A). The hypocotyl and root length has been normalized to the wildtype which grew at the same conditions. Data were averaged between three replicates with at least 33 seedlings per line and treatment. Shown are mean values and standard deviations. Student's ttest was used to calculate statistical significance. Stars above bars reveal statistical significance of difference in comparison to the wildtype. *p<0.05; **p<0.01; ***p<0.001

For the investigation of hypocotyl and root growth during osmotic stress conditions the hypocotyl and root length of the wildtype which grew at the same conditions like the *ahk1* knock down lines was defined as 100%. After growth on media which was supplemented with different concentrations of mannitol hypocotyl and root length was in general decreased (data not shown). Furthermore, *ahk1-3* and *ahk1-4* showed the previously detected reduced hypocotyl (fig. 4.16 B) and root length (fig. 4.16 C) on control conditions whereas *ahk1-3/35S::AHK1-GFP* did not show an altered hypocotyl and root length in comparison to the wildtype. After growth on media which was supplemented with 50mM, 100mM and 300mM mannitol no difference of hypocotyl length could be detected for these lines except for the decreased hypocotyl length of *ahk1-3* after growth on 100mM mannitol. In contrast, a decreased root length could be revealed for *ahk1-3* after growth on 100mM and 300mM mannitol, for *ahk1-4* after growth on 50mM mannitol and for *ahk1-3/35S::AHK1-GFP* after growth on 300mM mannitol. Hence, osmotic stress seems to slightly increase the hypocotyl and root elongation in etiolated seedlings of *ahk1-3* and *ahk1-4*.

The reproducibility of this assay enabled to check, whether temperature alters the hypocotyl and root growth and might be one factor which led to the opposing results in the root growth assay. At 20°C three day old etiolated seedlings of *ahk1-3* and *ahk1-4* revealed shorter roots and hypocotyls in comparison to the wildtype whereas *ahk1-3/35S::AHK1-GFP* showed wildtype-like hypocotyl and root length. At 28°C a general increase of hypocotyl length could be observed whereas root length was decreased. The *ahk1* knock down lines *ahk1-3* and *ahk1-4* showed wildtype-like hypocotyl and root length at this elevated temperature (fig. 4.17). This indicates an influence of the temperature on the elongation of roots and hypocotyls.

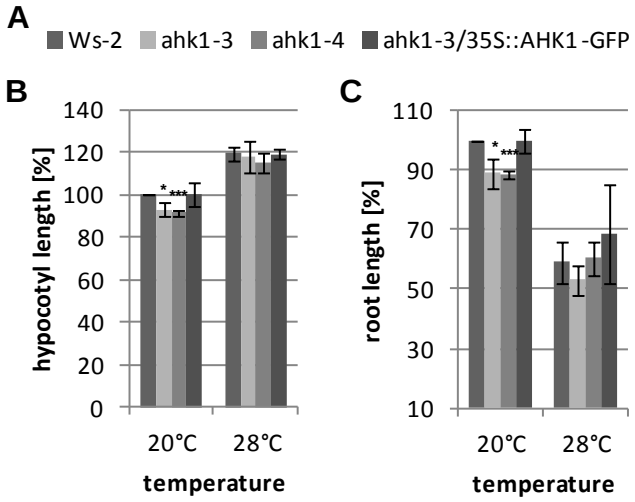



figure 4.17: Hypocotyl and root length of etiolated seedlings after growth at different temperatures
Hypocotyl (B) and root (C) length of 3d old etiolated seedlings of *ahk1-3*, *ahk1-4*, *ahk1-3/35S::AHK1-GFP* and their wildtype Ws-2 which grew at 20°C and 28°C after 2h light induction of germination. The color code for the lines in (B) and (C) is given in (A). Hypocotyl and root length has been normalized to the wildtype which has been grown at 20°C. Data were averaged between three replicates with at least 38 (20°C) or 5 (28°C) seedlings per line and treatment. Shown are mean values and standard deviations. Student's ttest was used to calculate statistical significance. Stars above bars reveal statistical significance of difference in comparison to the wildtype. *p<0.05; **p<0.01; ***p<0.001

To analyze whether the altered length of hypocotyls and roots in the *ahk1* knock down alleles depends on altered germination a germination assay was executed. The germination rate on 0.3M mannitol as well as on the control did not differ between the wildtype, *ahk1* knock down alleles and *ahk1-3/35S::AHK1-GFP* after five days at constant light conditions whereas the germination rate was generally lower on 0.3M mannitol than on the control (fig. 4.18). This is contradictory to the findings of Wohlbach *et al.* (2008) and Katharina Caesar but here it has to be noted that seeds were used which were not older than six months. For other experiments like for instance root growth assays it was observed that the germination especially of *ahk1* knock down lines was reduced when the seeds were older than six months (data not shown). Beside the germination rate the germination time was also not altered in this experiment (fig. 4.35).

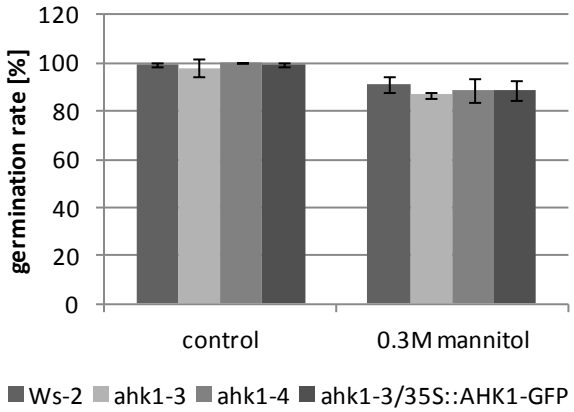



figure 4.18: Percentage of germination on media supplemented with 0.3M mannitol.
Germination rate after four days of stratification and five days growth at constant light at 20°C on half strength MS salts (control) and media supplemented with 0.3M mannitol. Mean values and standard deviation of three replicates with 50 seeds per line and condition. Student's t-test was used to calculate statistical significance. No statistical significant difference in the germination rate could be determined for these lines and these conditions.

#### 4.1.3.8 Influence of AHK1 on hormone metabolism and signaling

In the phosphoproteome of *ahk1-3* and the wildtype Ws-2 several phosphopeptides were quantified which were categorized into hormone metabolism or respective signaling (appendix A33, A34, A35). Additionally, other AHKs are known to be receptors for cytokinin and ethylene.

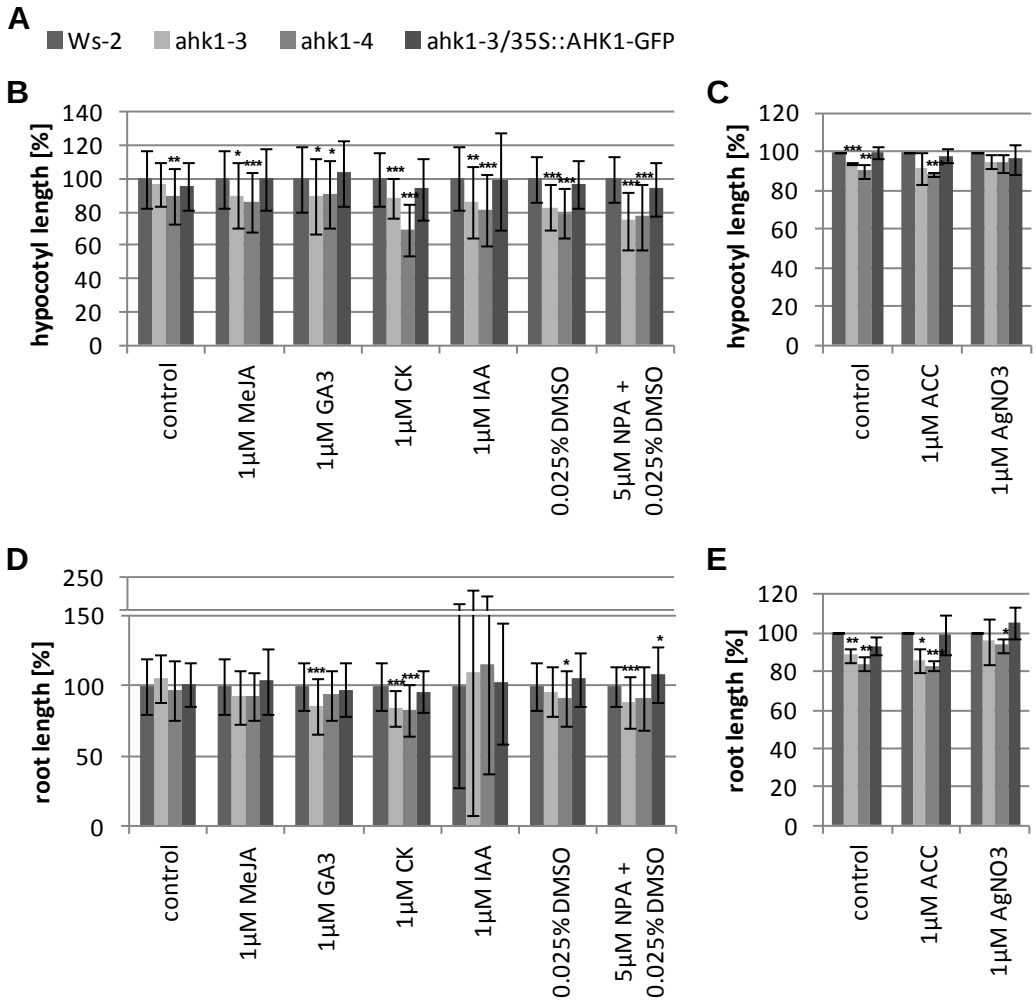



figure 4.19: Hypocotyl and root length of etiolated seedlings upon treatment with hormones or inhibitors of hormone biosynthesis or signaling
Hypocotyl (B, C) and root (D, E) length of seedlings of *ahk1-3*, *ahk1-4*, *ahk1-3/35S::AHK1-GFP* and their wildtype Ws-2 which grew in the dark for three days after 2h light induction of germination on control media and media which were supplemented with the hormones methyl-jasmonate (MeJA), gibberellic acid (GA3), kinetin (CK), indole-3-acetic acid (IAA), the ethylene precurser 1-aminocyclopropane-1-carboxylic acid (ACC), the inhibitor of ethylene signaling silver nitrate (AgNO3) as well as on the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA). As NPA was solved in DMSO, a DMSO control was added. The color code of the tested plant lines for (B), (C), (D) and (E) is given in (A). The hypocotyl and root length has been normalized to the respective wildtype. Data were averaged between one (B, D) or three (C, E) replicates with at least 37 seedlings per line and treatment. Shown are mean values and standard deviations. Student's ttest was used to calculate statistical significance. Stars above bars reveal statistical significance of difference in comparison to the wildtype. *p<0.05; **p<0.01; ***p<0.001

Therefore it was tested, whether *ahk1* knock down alleles show differences in root elongation of light-grown seedlings or in length of hypocotyls and roots in etiolated seedlings upon treatment with different hormones or inhibitors of hormone biosynthesis or signaling. One experiment with at least ten seedlings per line and treatment did not reveal any difference in root elongation between *ahk1-3* and

the wildtype Ws-2 upon treatment with indole-3-acetic acid (IAA), the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA), methyl-jasmonate (MeJA), salicylic acid (SA), abscissic acid (ABA), giberellic acid 3 (GA3), the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), the inhibitor of ethylene signal transduction silver nitrate ($AgNO_3$) and kinetin (CK) (appendix A27 D).
In etiolated seedlings the hypocotyl length of *ahk1-3* and *ahk1-4* was decreased in comparison to the wildtype after growth on MeJA, GA3, CK, IAA as well as after growth on NPA (fig. 4.19 B). The root length of the *ahk1* knock down alleles in the single experiment with hormones differed from the root length of the other experiments as they did not show a decrease after growth on control conditions. As well no difference between the root length of the wildtype, the *ahk1* knock down alleles and *ahk1-3*/*35S::AHK1-GFP* could be revealed for seedlings grown on MeJA and IAA. Though, a decreased root length was detected for *ahk1-3* grown on GA3, CK and NPA as well as for *ahk1-4* grown on CK and DMSO. In contrast to *ahk1-3*, *ahk1-3*/*35S::AHK1-GFP* showed an increased root length after growth on NPA (fig. 4.19 D). In three replicates with at least 37 seedlings per line and condition *ahk1-3* and *ahk1-4* again showed a reduced hypocotyl and root length on control conditions. Just *ahk1-4* showed a reduced hypocotyl length after growth on ACC, whereas after growth on $AgNO_3$ no difference was revealed between the wildtype and the *ahk1* knock down lines or *ahk1-3/35S::AHK1-GFP* (fig. 4.19 C). The root length was reduced after growth on ACC in *ahk1-3* and *ahk1-4,* after growth on $AgNO_3$ just in *ahk1-4* (fig. 4.19 E).

#### 4.1.3.9 Role of AHK1 in auxin signaling

Application of IAA increased the shortening of hypocotyls in etiolated seedlings of the *ahk1* knock down alleles in comparison to the respective wildtype. In addition, several peptides were indentified in the phosphoproteome of *ahk1-3* and Ws-2 which are connected to auxin metabolism, transport and signaling. The phosphorylation patterns as well as the $log_2$-values of some of these quantified phosphopeptides are listed in table 4.4.

table 4.4: Quantified phosphopeptides of proteins of auxin metabolism, transport and signaling
$log_2$-values for the ratio of quantified peptides in *ahk1-3* and the wildtype (wt) Ws-2 in the experiments with a reciprocal metabolic labeling (met. labeling) experimental design or without labeling (no labeling) after 10min treatment with 0.3M mannitol (+man) or mock respectively (-man). For met. labeling +man $log_2$-values of the two reciprocal replicates are shown. The phosphorylated residue is highlighted with a subsequent (ph). (ac) reveals acetylation, (ox) oxidation. The "x" shows that the $log_2$-value could not be calculated due to a not quantified peptide for the nominator or denominator. "-" marks no quantification. Data obtained from Waltraud X. Schulze.

| accession | name | phosphopeptide | $log_2$-values (*ahk1-3*/wt) | | | | |
|---|---|---|---|---|---|---|---|
| | | | met. labeling | | | no labeling | |
| | | | +man | | -man | +man | -man |
| AT3G04730 | IAA16 | TYQDLSNALS(ph)K | -21.57 | -8.31 | - | - | - |
| AT1G34410 | ARF21 | LFGVT(ph)LDTPPM(ox)IK | 4.23 | 3.12 | - | - | - |
| AT1G70940 | PIN3 | M(ox)LIM(ox)EQFPETAASIVS(ph)FK | - | - | - | -0.42 | x |
| | | PSNLTGAEIYS(ph)LST(ph)TPR | - | - | - | x | 0.23 |
| AT1G23080 | PIN7 | PSNLTGAEIYS(ph)LNT(ph)TPR | - | - | - | -0.64 | 0.34 |
| AT1G15750 | TPL | APS(ph)PVNNPLLGGIPK | - | - | 0.15 | 0.14 | 0.29 |
| AT1G68370 | ARG1 | AQGDESKGDGDS(ph)AGEEGGTENR | - | - | - | 1.16 | 0.50 |
| | | AQGDESKGDGDS(ph)AGEEGGTENRDK | - | - | - | 0.52 | -0.08 |

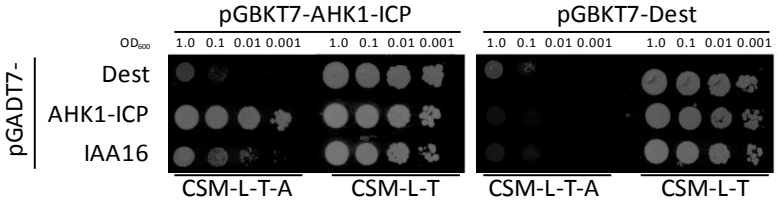



figure 4.20: AHK1-ICP interacts with IAA16 in yeast.
The reporter yeast strain pJ69-4A was co-transformed with the *BD*-fusion contruct of *AHK1-ICP* (*pGBKT7-AHK1-ICP*) and the *AD*-fusion constructs of *AHK1-ICP* (*pGADT7-AHK1-ICP*) and *IAA16* (*pGADT7-IAA16*) respectively. The empty destination-vectors (*pGBKT7-Dest*, *pGADT7-Dest*) were used as control. For the interaction test the respectively transformed yeast was plated on the auxotrophy medium CSM-Leu$^-$-Trp$^-$-Ade$^-$ (CSM-L-T-A) as well as on the growth control medium CSM-Leu$^-$-Trp$^-$ (CSM-L-T) and grown for four days at 28°C. The detection of expressed protein is shown in appendix A23.

IAA16 is differentially phosphorylated at Ser150 which is located in domain III (Korasick *et al.*, 2014). Domain III is involved in the mediation of homo- and heterodimer formation with ARFs. Unfortunately ARF21 could not be cloned to test whether IAA16 interacts with ARF21 and whether this interaction is influenced by the differential phosphorylation. Nevertheless, in a yeast two-hybrid assay an interaction of the intracellular part of AHK1 (AHK1-ICP) comprising aa440-1207 with IAA16 could be revealed (fig. 4.20). To verify this interaction *AHK1-GFP* and *RFP-IAA16* were transiently expressed under the control of the *CaMV 35S-promoter* in *Nicotiana benthamiana*. AHK1-GFP localizes to the plasma membrane and vesicle-like structures which were described in 4.1.1.2. whereas RFP-IAA16 localizes to the nucleus (fig. 4.21) independently from AHK1 (appendix A28). To analyze, whether the phosphorylation has any influence on the localization or interactions of IAA16, Ser150 was mutated to Ala to mimick the not phosphorylated state as well as to Glu to mimick the phosphorylated state of IAA16. The constructs are ready for further investigation.

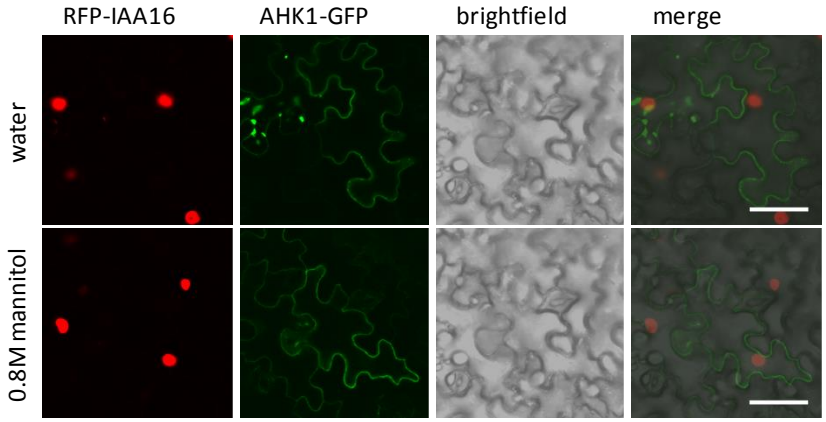



figure 4.21: Localization of RFP-IAA16 and AHK1-GFP
Transient co-expression of I*AA16* tagged with a N-terminal *RFP* (*RFP-IAA16*, red) and *AHK1* tagged with a C-terminal *GFP* (*AHK1-GFP*, green) in *N. benthamiana* reveals in confokal microscopy that RFP-IAA16 localizes to the nucleus in general and during osmotic stress applied with 0.8M mannitol whereas AHK1-GFP localizes to the plasmamembrane and vesicle-like compartments as they were described in 4.1.1.2. The expression of *AHK1-GFP* and *RFP-IAA16* was regulated by *CaMV 35S-promoter* each. The scale is 50µm.

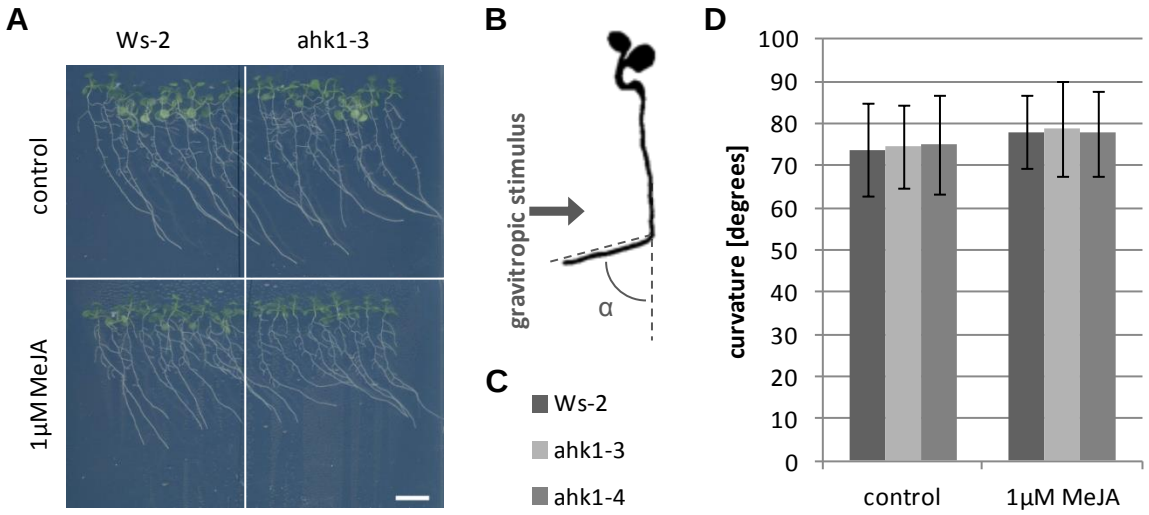



figure 4.22: Gravitropic growth is not influenced in *ahk1* knock down mutants.
(A) Twelve day old Ws-2 and *ahk1-3* seedlings which have been transferred to control media and media supplemented with 1µM methyl-jasmonate (MeJA) four days after germination. The bar gives 1cm. (B) Scematic view of the gravitropic response. α specifies the curvature. (C) Color code for Ws-2, *ahk1-3* and *ahk1-4* in (D). (D) Curvature upon an altered gravitropic stimulus three days after germination. The roots were scored two days later. Shown are the mean values and standard deviations of one experiment with at least 90 seedlings per line and treatment.

In the phosphoproteome ARG1 is more phosphorylated in *ahk1-3* after 10min treatment with 0.3M mannitol. ARG1 as well as PIN3 and auxin are involved in the response of plants to gravity (Sedbrook *et al.*, 1999; Harrison and Masson, 2008). Additionally, in the previous study of AHK1-dependent root growth *ahk1-3* seemed to be influenced in gravitropic growth upon treatment with 1µM MeJA (fig. 4.22 A). In a gravitropic growth assay this was further tested. Therefore, seedlings growing on ½ MS-agar with and without supplementation of 1µM MeJA were exposed to a gravitropic stimulus coming from 90° left and the curvature of the root was measured after additional two days of growth (fig. 4.22 B). A difference in the curvature of gravitropic growth could not be detected (fig. 4.22 D).

An alteration in the gravitropic stimulus leads to auxin redistribution in the root and therefore to the induction of lateral root development whereas PIN3 and PIN7 which were quantified in the phosphoproteome of *ahk1-3* and the wildtype Ws-2 play central roles in the redistribution of auxin (Marhavý *et al.*, 2013). Therefore the initiation of lateral root development was analyzed in *ahk1* knock down lines in three different ecotypes. 18h and 42h after gravitropic induction of lateral root development the lateral root primordia (LRP) were analyzed and classified into the stages (fig. 4.23) how they were described for instance by De Smet *et al.* (2015).

18h after the gravitropic induction of lateral root development LRPs in all analyzed lines could be categorized to stage 0, I or II, 42h after the gravitropic induction LRPs in all analyzed lines could be categorized to the stages IV to VIII. 18h after gravitropic induction approximately 50% of Nos-0 and *ahk1-1* showed either stage I or stage II LRPs, whereas about 40% of seedlings of *ahk1* knock down alleles in the Ws-2 and Col-0 ecotype and their respective wildtypes showed stage I LRPs and about 60% of the seedlings stage II LRPs. Just few seedlings of *ahk1-1* and *ahk1-6* did not show any LRP

18h after gravitropic induction. Besides, less seedlings of *ahk1-1* had stage II LRPs in comparison to the wildtype.

42h after the gravitropic induction there was in general no difference in the amount of plants with LRPs in the respective stages between the *ahk1* knock down lines and their respective wildtypes in the Nos-0 and Col-0 ecotype. This was also true for the Ws-2 ecotype except that there were more *ahk1* knock down seedlings with stage VI LRPs than in the wildtype. The number of seedlings with LRPs in the respective stage was different between the three ecotypes. 42h after gravitropic induction seedlings of Nos-0 and *ahk1-1* mainly had LRPs of the stage VII, less plants showed LRPs in the stages V, VI and VIII and just few had LRPs which were categorized to stage IV. This was also true for the Ws-2 ecotype with the difference that in the Ws-2 ecotype approximately the same number of LRPs could be categorized to stage VII and stage VIII. 42h after gravitropic induction seedlings of the Col-0 ecotype did not have stage IV LRPs. The seedlings showed mainly stage V and stage VIII LRPs and less stage VI and stage VII LRPs whereas the amount of plants with LRPs categorized to stage V and VIII as well as to stage VI and VII was approximately the same.

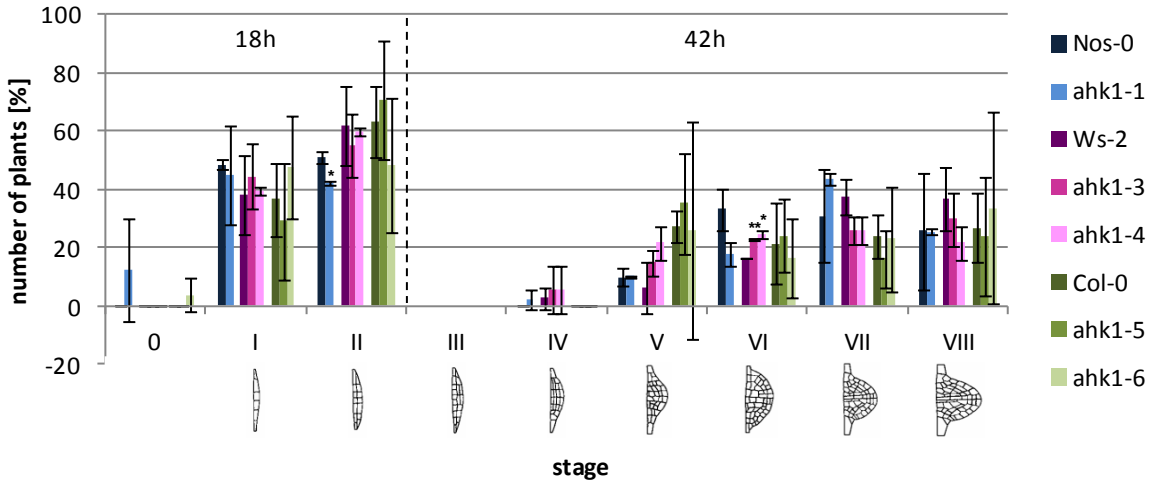



figure 4.23: Lateral root development of *ahk1* knock down mutants.
Stages of the lateral root development in *ahk1* knock down mutants and their respective wildtypes in the ecotypes Nos-0 (blue), Ws-2 (pink) and Col-0 (green) 18h (stage I-III) and 42h (stage IV-VIII) after the change of the gravitropic stimulus to 90° to the side. Shown are the mean values and standard deviation of two independent experiments with a minimum of 13 seedlings per line. The illustration of the stages beyond the axis has been taken from De Smet *et al.* 2015.

42h after the gravitropic induction there was in general no difference in the amount of plants with LRPs in the respective stages between the *ahk1* knock down lines and their respective wildtypes in the Nos-0 and Col-0 ecotype. This was also true for the Ws-2 ecotype except that there were more *ahk1* knock down seedlings with stage VI LRPs than in the wildtype. The number of seedlings with LRPs in the respective stage was different between the three ecotypes. 42h after gravitropic induction seedlings of Nos-0 and *ahk1-1* mainly had LRPs of the stage VII, less plants showed LRPs in the stages V, VI and VIII and just few had LRPs which were categorized to stage IV. This was also true for the Ws-2 ecotype with the difference that in the Ws-2 ecotype approximately the same number of LRPs could be categorized to stage VII and stage VIII. 42h after gravitropic induction seedlings of the Col-0 ecotype did not have stage IV LRPs. The seedlings showed mainly stage V and stage VIII LRPs and less stage

VI and stage VII LRPs whereas the amount of plants with LRPs categorized to stage V and VIII as well as to stage VI and VII was approximately the same.

As the number of plants with a LRP in a certain stage was always similar for at least three *ahk1* knock down alleles in at least two different ecotypes and as there was no statistical significant difference there is no indication for AHK1-dependent changes in lateral root development.

Osmotic stress highly influences the root system architecture (Duan *et al.* 2013). As big differences could be revealed in the phosphoproteome of *ahk1-3* and the wildtype after 10min of mannitol treatment it was interesting whether *ahk1* knock down alleles show differences in hydrotropic growth and the root system architecture.

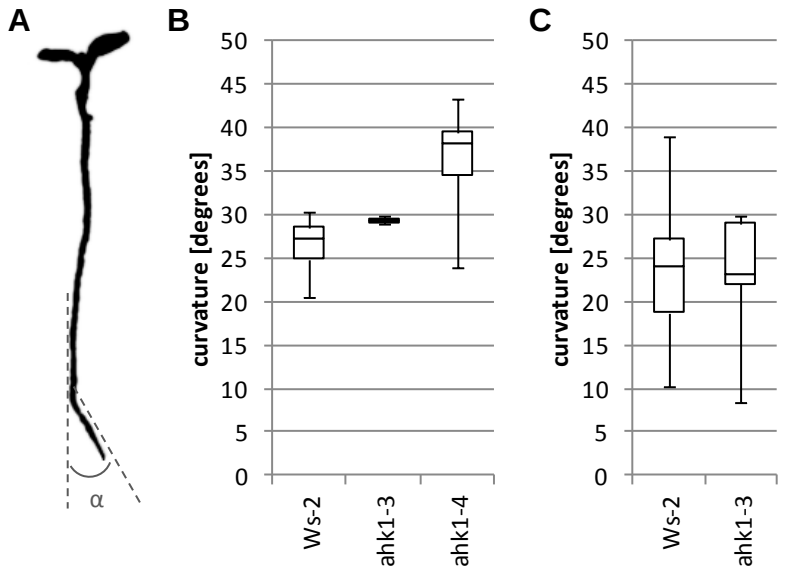



figure 4.24: Hydrotropic response of *ahk1* knock down mutants
(A) Scematic view of the hydrotropic response. α specifies the curvature. (B) Hydrotropic response of wildtype (Ws-2) and *ahk1* knock down mutants. Shown are results of four experiments with 35 seedlings per line and experiment. No statistical significant difference could be shown using student's t-test. (C) Hydrotropic response of Ws-2 and *ahk1-3* in eleven experiments with 35 seedlings per line and experiment. Using student's t-test no statistical significant difference could be shown.

The hydrotropic growth was tested with the use of sorbitol. In this assay, four day old seedlings were exposed to a diagonal water potential gradient which forces the roots to grow towards areas with higher water potential neglecting gravitropic growth (fig. 4.24 A). The greater the angle or curvature was the higher was the sensitivity and adaptability to the recognition of water potential. For *ahk1* knock down lines the variation of the curvature, was very high (fig. 4.24 B, C). Results of four experiments with 35 seedlings per *Arabidopsis thaliana* line show a not significant tendency for *ahk1-3* and *ahk1-4* to show a greater curvature than the wildtype indicating that AHK1 negatively influences the sensitivity to high water potential or positively influences the sensitivity to low water potential (fig. 4.24 B). After 11 repeats of this assay the variation of the hydrotropic curvature of *ahk1-3* increases and the previous tendency of the greater curvature was lost completely (fig. 4.24 C). This indicates that AHK1 does not contribute to hydrotropic growth. In a cooperation with Christa Testerink (Plant Physiology, Swammerdam Institute of Life Sciences, University of Amsterdam, NL) Dorota Kawa, her Ph.D. student, performed a halotropism assay which basically is a hydrotropic growth assay with NaCl as well as a Root System Architecture (RSA) assay to further characterize and investigate the root growth of *ahk1* knock down lines in response to salt stress. For the halotropism assay it was impossible to draw any conclusions as Ws-2 as well as *ahk1-3* and *ahk1-4* were skewing too much at control

conditions. In the presence of salt the skewing was less for all lines but no AHK1-dependent difference could be detected (appendix A29).

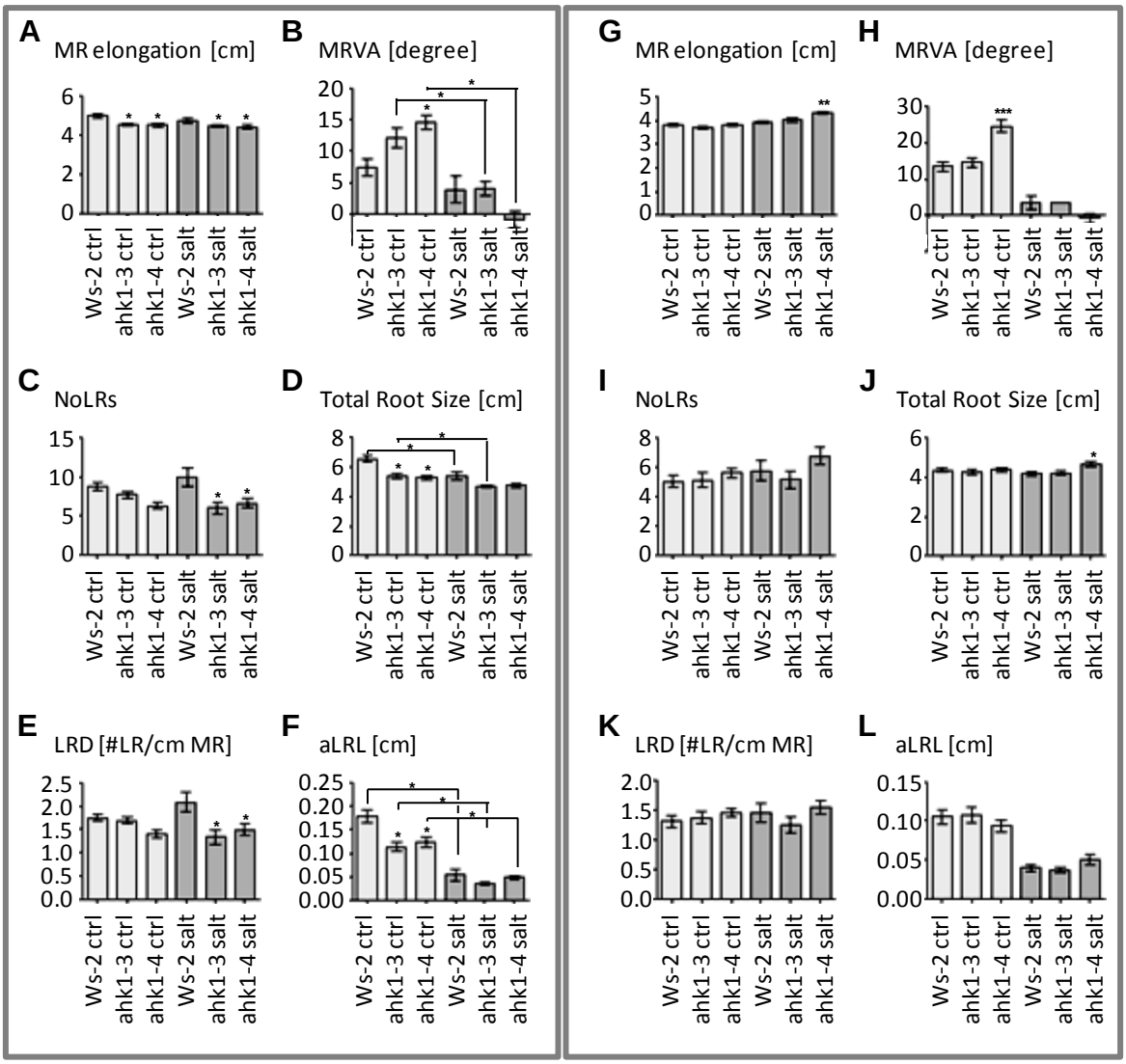



figure 4.25: Results of two experiments of the Root System Architecture assay which were executed by Dorota Kawa, a Ph.D. student of Christa Testerink.
Root System Architecture (RSA) traits of Ws-2 (wildtype), *ahk1-3* and *ahk1-4* on control conditions (ctrl, light grey bars) and in presence of 75mM NaCl (salt, dark grey bars) in replicate 1 (A-F) and replicate 2 (G-L). RSA parameters are the elongation of the main root (MR) after the transfer of seedlings to control or salt conditions (A, G), the main root vector angle (MRVA, B, H), the number of lateral roots (NoLRs, C, I), the total root size (D, J), the lateral root density (LRD, E, K) as well as the average lateral root (LR) length (aLRL, F, L). The parameters were quantified four days after transfer for plants grown on control plates and six days after transfer for plants grown on salt stress. Statistical comparisons were done by one-way ANOVA (post hoc Tuckey's test). Significant differences to the wildtype are indicated with stars directly above the bars, stars above brackets give the statistical significance of the two indicated lines. *p<0.05; **p<0.01; ***p<0.0001.

The parameters investigated in the RSA assay were the main root (MR) elongation, the main root vector angle (MRVA), the number of lateral roots (NoLRs), the total root size, the lateral root (LR) density (LRD) and the average lateral root length (aLRL). The assay was repeated twice. The results of both replicates for each parameter were contradictory for the *ahk1* knock down lines in comparison

to the wildtype at control conditions as well as during salt stress. Whereas in the first experiment the MR elongation was reduced for *ahk1-3* and *ahk1-4* at control conditions as as well as on salt stress (fig. 4.25 A), in the second experiment there was no difference or even an increase of the main root elongation (fig. 4.25 G). The MRVA was generally reduced for all lines at salt stress conditions in comparison to control conditions. It was the only parameter which revealed the same result in both experiments, showing an increased MRVA for *ahk1-4* at control conditions (fig. 4.25 B, H). Further, in the first experiment for *ahk1-3* and *ahk1-4* the number of lateral roots and the LRD (fig. 4.25 E) was reduced during salt stress (fig. 4.25 C), the total root size (fig. 4.25 D) as well as the aLRL was reduced at control conditions (fig. 4.25 F). In the second experiment no difference between wildtype and *ahk1* knock down alleles could be observed for neither NoLRs (fig. 4.25 I), total root size (fig. 4.25 J), LRD (fig. 4.25 K) nor aLRL (fig. 4.25 L). During salt stress in both experiments the aLRL was reduced for all lines (fig. 4.25 F, L).

Furthermore it could be shown in twelve day old seedlings after eight days of mannitol treatment, that there is no AHK1-dependent difference in the number of lateral roots during control or osmotic stress conditions (appendix A30) whereas *ahk1-3* as well as *ahk1-3*/*35S::AHK1-GFP* showed a reduced number of lateral roots on 0mM mannitol. As this could not be shown for *ahk1-4* this seems to be an allele specific effect.

#### 4.1.3.10 Calcium signaling and ion channels

The analysis of the phosphoproteome of *ahk1-3* and Ws-2 revealed many phosphopeptides which are involved in calcium signaling or ion transport (appendix A33, A34, A35).

Therefore it was tested whether seedlings of *ahk1-3* show an altered root elongation in comparison to the wildtype upon treatment with different concentrations of $CaCl_2$.

One experiment with at least ten seedlings per line and treatment showed a decreased root elongation of *ahk1-3* on 1mM and 10mM $CaCl_2$ but not on 0.1mM and 100mM $CaCl_2$ (appendix A27 B). To verify the influence of calcium on the root elongation the experiment has to be repeated including *ahk1-4* and *ahk1-3/35S::AHK1-GFP*.

Even if the difference of root elongation upon increased exogenous calcium levels is not reproducible there might be AHK1-dependent differences in the calcium influx or the intracellular calcium signature in cells. Therefore *pGPTVII-Bar-U-RGECO1* of Karin Schuhmacher was stably transformed into the *ahk1* knock down alleles in all ecotypes and their respective wildtypes. Unfortunately this did not work and has to be repeated. In addition, *ahk1-5* and *ahk1-6* were crossed with Col-0/R-GECO1 of Karin Schuhmacher to obtain *ahk1* knock down alleles which can be investigated in regard to calcium influx and calcium signature. F1 seeds could already be obtained but have to be progenated for further investigation.

The number of ion channels and transporters which were quantified in the phosphoproteome of *ahk1-3* and Ws-2 arose the question whether *ahk1* knock down alleles reveal an altered ion content. To test this, 14 day old seedlings which were grown in liquid culture were treated for 10min with 0.3M mannitol or mock, harvested and dried. The content of sodium, potassium and calcium ions in the different plant lines was determined with the use of a flame photometer. The flame photometric analysis of dried plant material did not reveal any differences between *ahk1-3*, *ahk1-4*, *ahk1-3*/*35S::AHK1-GFP* and the

wildtype Ws-2 in regard to potassium and calcium content (fig. 4.26 B, D). The same is true for the sodium content exept for *ahk1-3* which showed a slight increase in sodium content under control conditions (fig. 4.26 C). Furthermore *ahk1-3* shows a drop in potassium and sodium content after 10min treatment with 0.3M mannitol (fig. 4.26 B, C). This was also observed for *ahk1-3*/*35S::AHK1-GFP* but for the calcium content (fig. 4.26 D). As the increase or drop of the ion content was observed in just one allele respectively the differences might be due to genotype specific factors and might not depend on AHK1.

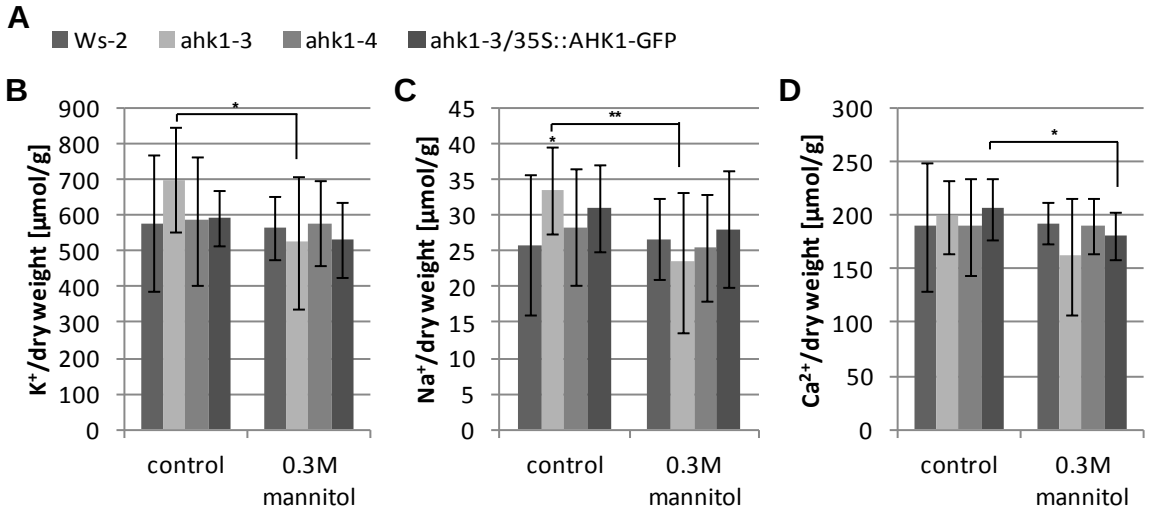



figure 4.26: Ion content of *ahk1* knock down lines
Potassium- (B), sodium- (C) and calcium-ion (D) content per dry weight of 14d old A*rabidopsis thaliana* seedlings of the *ahk1* knock down lines *ahk1-3* and *ahk1-4*, *ahk1-3*/*35S::AHK1-GFP* and the respective wildtype. The plants were grown in liquid culture and treated with 0.3M mannitol or mock for 10min. (A) gives the color code for B, C, and D. The diagrams show mean values and standard deviations of one experiment with 9-12 biological replicates per line and treatment. Student's t-test was used to calculate statistical significance. Stars directly above the bars show the statistical significance of difference to the wildtype. Stars above brackets reveal the significance of difference between the two marked plant lines. *p<0.05; **p<0.01

#### 4.1.3.11 Effect of AHK1 on aquaporins

The analysis of the phosphoproteome in *ahk1-3* and Ws-2 after 10min treatment with 0.3M mannitol, revealed 15 phosphopeptides originating from seven aquaporins whereas different phosphorylation patterns per peptide were detected (table 4.5). Some of these peptides with the respective phosphorylation pattern have already been identified by Wu *et al.* (2013) in the analysis of the phosphoproteome of the *sirk1* T-DNA insertional mutant and could be correlated to the altered swelling behavior of *sirk1* protoplasts in comparison to the wildtype when exposed to hypo-osmolar medium. This was not the case for *ahk1-3*, *ahk1-4* or *ahk1-3*/*35S::AHK1-GFP* (fig. 4.27), neither in protoplasts of seedling shoots (fig. 4.27 A) nor in protoplasts of seedling roots (fig. 4.27 B). The volume of the protoplasts before and after swelling did not show any genotype specific differences as well, neither in protoplasts of shoots (fig. 4.27 C) nor in protoplasts of seedling roots (fig. 4.27 D).

table 4.5: Quantified phosphopeptides of plasma membrane intrinsic proteins
$log_2$-values for the ratio of quantified peptides in *ahk1-3* and the wildtype (wt) Ws-2 in the experiments with a reciprocal metabolic labeling (met. labeling) experimental design or without labeling (no labeling) after 10min treatment with 0.3M mannitol (+man) or mock respectively (-man). For met. labeling +man $log_2$-values of the two reciprocal replicates are shown.The phosphorylated residue is highlighted with a subsequent (ph). (ac) reveals acetylation, (ox) oxidation. The "x" shows that the $log_2$-value could not be calculated due to a not quantified peptide for the nominator or denominator. "-" marks no quantification. Underlined values revealed statistical significance with $p<0.05$. Data obtained from Waltraud X. Schulze.

| accession | name | phosphopeptide | $log_2$-values (*ahk1-3*/wt) | | | | |
|---|---|---|---|---|---|---|---|
| | | | met. labeling | | | no labeling | |
| | | | +man | | -man | +man | -man |
| AT3G53420 | PIP2A | SLGS(ph)FRS(ph)AANV | - | - | 0.26 | 0.71 | 0.72 |
| | | SLGS(ph)FRSAANV | -0.50 | -1.70 | 0.11 | - | - |
| | | SLGSFRS(ph)AANV | - | - | -0.57 | - | - |
| AT2G37170 | PIP2B | SLG(pS)FRSAANV | -14.94 | -1.70 | - | - | - |
| AT3G54820 | PIP2D | ALGS(ph)FRS(ph)QPHV | - | - | - | 1.63 | -0.22 |
| AT2G39010 | PIP2E | S(ph)QLHELHA | - | - | - | -0.18 | 1.12 |
| | | TKDELTEEES(ph)LSGK | - | - | - | -1.72 | 0.92 |
| AT5G60660 | PIP2F | ALGS(ph)FGS(ph)FGSFR | - | - | - | -0.37 | 0.57 |
| | | ALGSFGS(ph)FGS(ph)FR | - | - | - | -0.12 | 0.70 |
| | | ALGSFGS(ph)FGSFR | - | - | 0.13 | - | - |
| | | ALGSFGSFGS(ph)FR | - | -1.55 | x | - | - |
| AT4G35100 | PIP3A | ALGS(ph)FRS(ph)NATN | - | - | 0.13 | - | - |
| | | ALGS(ph)FRSNATN | -21.23 | -12.98 | 0.19 | -0.33 | -0.14 |
| | | ALGSFRS(ph)NATN | - | - | 0.49 | - | - |
| AT2G16850 | PIP3B | ALAS(ph)FRS(ph)NPTN | - | - | - | -0.22 | 0.71 |

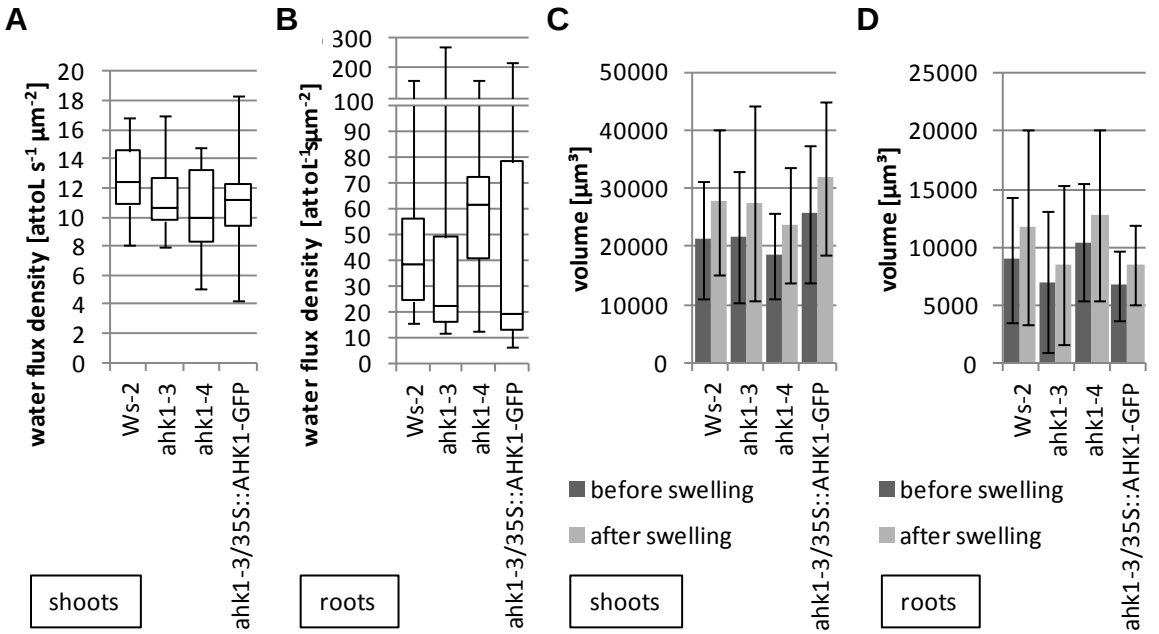



figure 4.27: Protoplast swelling assay did not reveal AHK1-dependent changes in the water flux density. Protoplasts of shoots (A, C) and roots (B, D) of four day old seedlings were exposed to hypo-osmolar medium. The swelling of the protoplasts was recorded by video-microscopy. The change of volume per time was measured and the water flux density calculated. Shown are box plots of water flux densities of at least 12 individual protoplasts per line and tissue (A, B) as well as the mean volume of the protoplasts of shoots (C) and roots (D) before and after swelling and the respective standard deviations. Student's t-test did not reveal any statistical significant differences.

#### 4.1.3.12 AHK1-dependent phosphorylation of AHAs

The functional categorization of quantified phosphopeptides revealed several v- and p- ATPases (appendix A33, A34, A35) comprising also plasma membrane $H^+$-ATPases (AHAs) (table 4.6). AHAs contribute to several developmental and adaptive processes in plants. Therefore it was interesting whether the AHA activity is changed in *ahk1-3* in comparison to the wildtype.

table 4.6: Quantified phosphorylated peptides of AHAs
Shown are the $log_2$-values for the ratio of quantified peptides in *ahk1-3* and the wildtype (wt) Ws-2 in the experiments with a reciprocal metabolic labeling (met. labeling) experimental design or without labeling (no labeling) after 10min treatment with 0.3M mannitol (+man) or mock respectively (-man). For met. labeling +man $log_2$-values of the two reciprocal replicates are shown.The phosphorylated residue is highlighted with a subsequent (ph). (ac) reveals acetylation, (ox) oxidation. The "x" shows that the $log_2$-value could not be calculated due to a not quantified peptide for the nominator or denominator. "-" marks no quantification. Underlined values revealed statistical significance with $p<0.05$. Data obtained from Waltraud X. Schulze.

| accession | name | phosphopeptide | $log_2$-values (*ahk1-3*/wt) | | | | |
|---|---|---|---|---|---|---|---|
| | | | met. labeling | | | no labeling | |
| | | | +man | | -man | +man | -man |
| AT2G18960 | AHA1 | EDVNIFPEKGS(ph)YR | - | - | - | 0.20 | 0.82 |
| | | T(ph)LHGLQPK | - | - | - | 0.34 | 0.09 |
| | | GLDIDTAGHHYT(ph)V | 1.00 | 0.77 | -0.42 | 0.08 | -0.64 |
| | | (ac)S(ph)GLEDIKNETVDLEK | - | - | - | -0.47 | x |
| AT4G30190 | AHA2 | GLDIETPSHYT(ph)V | -9.76 | -3.70 | -0.53 | 0.73 | 0.47 |
| | | EAVNIFPEKGS(ph)YR | - | - | - | -0.29 | 0.22 |
| | | LKGLDIETPSHYT(ph)V | - | - | -0.60 | - | - |
| AT5G57350 | AHA3 | LGMGS(ph)NMYPS(ph)SSLLGK | 2.34 | 3.08 | - | - | - |
| AT5G62670 | AHA11 | GLDIETIQQAYT(ph)V | - | - | - | 0.23 | 0.23 |
| | | LKGLDIETIQQAYT(ph)V | - | - | - | -0.87 | x |

The AHA activity was tested by Waltraud X. Schulze in 14 day old seedlings which were grown contemporaneously with the not labeled samples for the analysis of the phosphoproteome of *ahk1-3* and the wildtype Ws-2 in liquid culture and treated with 0.3M mannitol or mock. Therefore, the change of supplemented inorganic phosphate was measured in protein extracts how it was described in Lanzetta *et al.* (1979).

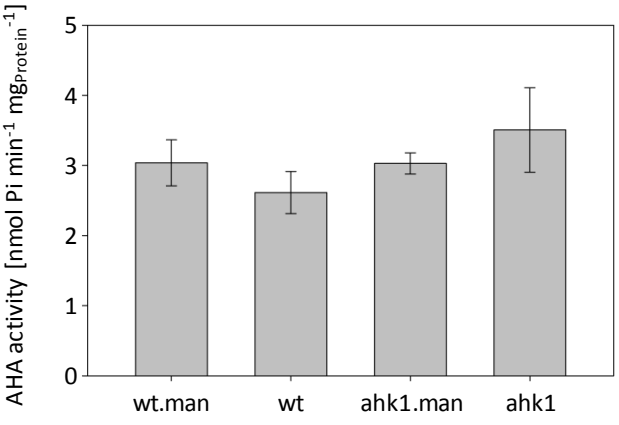



figure 4.28: AHA activity in *ahk1-3* and the wildtype Ws-2 after mannitol treatment.
AHA activity in protein extracts was determined by the measurement of the change of inorganic phosphate which was added to the protein extracts. As control for AHA-independent changes of inorganic phosphate the inhibitors $Na_3VO_4$, EDTA, $NaN_3$ and Bafilomycin A1 were added. Proteins were extracted from 14 day old seedlings of Ws-2 (wt) and *ahk1-3* (ahk1) which were grown in liquid culture and treated with mock or 0.3M mannitol (.man) which grew contemporaneously to the seedlings for the non-labeling phosphoproteomic approach. Shown are mean values and standard deviations of two technical replicates.

As control for AHA-independent changes of concentrations of inorganic phosphate $Na_3VO_4$ was added as inhibitor for AHAs, EDTA as inhibitor for $Ca^{2+}$-ATPases, $NaN_3$ as inhibitor for ATPases and

Bafilomycin A1 as inhibitor for V-ATPases. Neither for Ws-2 nor for *ahk1-3* after mock or mannitol treatment a difference in AHA activity could be revealed (fig. 4.28).

AHAs influence the pH within the plant and in the outside. Additionally, Kang *et al.* (2012), Koyama *et al.* (2001) and Zhou *et al.* (2010) suggested that the pH of the growth substrate influences root growth whereas low pH amongst others disrupts calcium processes and high pH increases the disassembly of microfilaments. Choi *et al.* (2014) and Cao *et al.* (2014) suggest extracellular ATP as central signaling molecule in plant stress responses. In addition, ATP is the substrate for ATPases. Therefore it was tested whether etiolated seedlings of *ahk1* knock down alleles reveal differences in hypocotyl and root length after growth on media with different pH or ATP-supplementation.

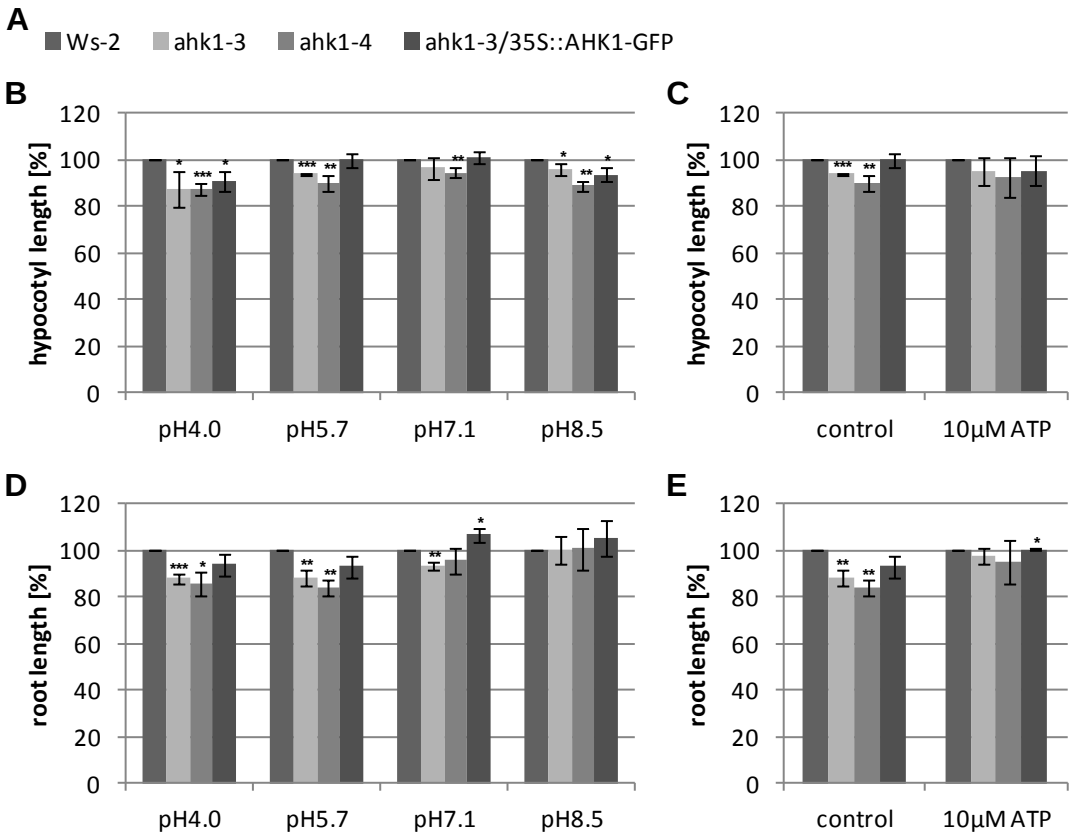



figure 4.29: Hypocotyl and root length of etiolated seedlings after growth on media with different pH or supplementation with ATP

Hypocotyl (B, C) and root (D, E) length of seedlings of *ahk1-3*, *ahk1-4*, *ahk1-3/35S::AHK1-GFP* and their wildtype Ws-2 which grew in the dark for three days after 2h light induction of germination on media with pH 4.0, pH 5.7, pH 7.1, pH 8.5 (B, D) or media supplemented with 10µM ATP (C, E). The color code for the lines in (B), (C), (D) and (E) is given in (A). The hypocotyl and root length has been normalized to the wildtype which grew at the same conditions. Data were averaged between three replicates with at least 35 seedlings per line and treatment. Shown are mean values and standard deviations. Student's ttest was used to calculate statistical significance. Stars above bars reveal statistical significance of difference in comparison to the wildtype. *p<0.05; **p<0.01; ***p<0.001

In three replicates with at least 35 seedlings per line and treatment *ahk1-3* and *ahk1-4* revealed reduced hypocotyl length after growth on media with pH 4.0, pH 5.7 and pH 8.5. Furthermore, reduced hypocotyl length was revealed for *ahk1-4* on media with pH 7.1 and for *ahk1-3/35S::AHK1-GFP* on media with pH 4.0 and pH 8.5 (fig. 4.29 B). A reduced root length could be detected for *ahk1-3* and

*ahk1-4* after growth on media with pH 4.0 and pH 5.7. *ahk1-3* showed a reduced, *ahk1-3/35S::AHK1-GFP* showed an increased root length on media of pH7.1 No difference in root length could be revealed on media with pH 8.5 (fig. 4.29 D). After growth on media with extracellular ATP no difference in the hypocotyl (fig. 4.29 C) and root length (fig. 4.29 E) of *ahk1-3*, *ahk1-4*, *ahk1-3/35S::AHK1-GFP* and their wildtype Ws-2 could be detected except the slight increase in root length of *ahk1-3/35S::AHK1-GFP*. Under control conditions *ahk1-3* and *ahk1-4* showed the reduced hypocotyl and root length like in previous experiments.

#### 4.1.3.13 AHK1, BAK1 and a putative superkomplex

Previous studies of Katharina Caesar which analyzed the function of AHK1 could show an interaction of AHK1 and BAK1 in mbSUS assays in *Saccharomyces cerevisiae* as well as in FRET-FLIM-studies using transient expression in *Nicotiana benthamiana*.

table 4.7: Quantified phophopeptides of brassinosteroid signaling and a putative supercomplex
Shown are the $log_2$-values for the ratio of quantified peptides in *ahk1-3* and the wildtype (wt) Ws-2 in the experiments with a reciprocal metabolic labeling (met. labeling) experimental design or without labeling (no labeling) after 10min treatment with 0.3M mannitol (+man) or mock respectively (-man). For met. labeling +man $log_2$-values of the two reciprocal replicates are shown. The phosphorylated residue is highlighted with a subsequent (ph). (ac) reveals acetylation, (ox) oxidation. The "x" shows that the $log_2$-value could not be calculated due to a not quantified peptide for the nominator or denominator. "-" marks no quantification. Underlined values revealed statistical significance with $p<0.05$. Data obtained from Waltraud X. Schulze.

| | | | $log_2$-values (*ahk1-3*/wt) | | | | |
|---|---|---|---|---|---|---|---|
| | | | met. labeling | | | no labeling | |
| accession | name | phosphopeptide | +man | | -man | +man | -man |
| AT4G39400 | BRI1 | S(ph)IEDGGFSTIEM(ox)VDM(ox)SIK | 12.82 | 5.99 | - | - | - |
| AT5G46330 | FLS2 | M(ox)NLT(ph)FISIGR | -19.63 | - | - | - | - |
| AT5G48380 | BIR1 | LK(t)F(s)V(s)DNRLVGPIPNFNQTLQFK | -47.46 | -14.91 | - | - | - |
| AT3G28450 | BIR2 | S(ph)GLTEVGVSGLAQR | - | - | - | 0,37 | 0,22 |
| AT5G40170 | RLP54 | S(ph)LVNC(t)(t)LK | -21.10 | - | - | - | - |
| AT3G23010 | RLP36 | IM(ox)DTFPFWLGS(ph)LPY(ph)LK | 10.00 | 10.00 | - | - | - |
| AT1G21210 | WAK4 | HIVSYFASAT(ph)K | 0.99 | 2.39 | - | - | - |
| AT1G21230 | WAK5 | IMGEERPS(ph)M(ox)K | 2.76 | <u>3.91</u> | - | - | - |
| AT1G15990 | CNGC7 | FIPLT(ph)SELK | 4.06 | - | - | - | - |
| AT2G23080 | CKA3 | AAE(s)(s)RLRTQ | -23.56 | - | - | - | - |
| AT4G35230 | BSK1 | SYS(ph)TNLAYTPPEYLR | - | - | - | 0,04 | -0,58 |
| AT5G46570 | BSK2 | TANLPSSDDPSAPNKPES(ph)VNGDQ VDQEIQNFK | - | - | - | -0,43 | x |
| AT3G54030 | BSK6 | SASVLES(ph)PDIENGGK | - | - | - | <u>x</u> | x |
| AT5G41260 | BSK8 | S(ph)NPDVTGLDEEGR | - | - | - | 0,20 | <u>1,08</u> |
| | | S(ph)NPDVTGLDEEGRGESNDLPQFR | - | - | - | -0,93 | <u>-1,07</u> |
| | | SYS(ph)TNLAFTPPEYLR | - | - | - | 1,07 | -1,93 |
| AT4G03080 | BSL1 | QLS(ph)LDQFQNESR | - | - | - | 2,31 | 0,87 |
| AT1G08420 | BSL2 | LIHPLPPALS(ph)SPETSPER | - | - | - | 0,45 | 0,27 |
| | | LVLFGGATALEGNSGGTGT(ph)PTSA GSAGIR | - | - | - | -1,22 | x |
| | | QLS(ph)IDQFENEGR | - | - | - | 0,27 | 0,31 |
| | | QLS(ph)IDQFENEGRR | - | - | - | x | x |
| | | QM(ox)S(ph)INSVPK | - | - | - | x | 0,80 |
| AT3G19820 | DWF1 | (ac)S(ph)DLQTPLVRPK | - | - | - | 0,49 | 0,84 |
| | | (ac)M(ox)SDLQT(ph)PLVR | - | 0.12 | - | - | - |

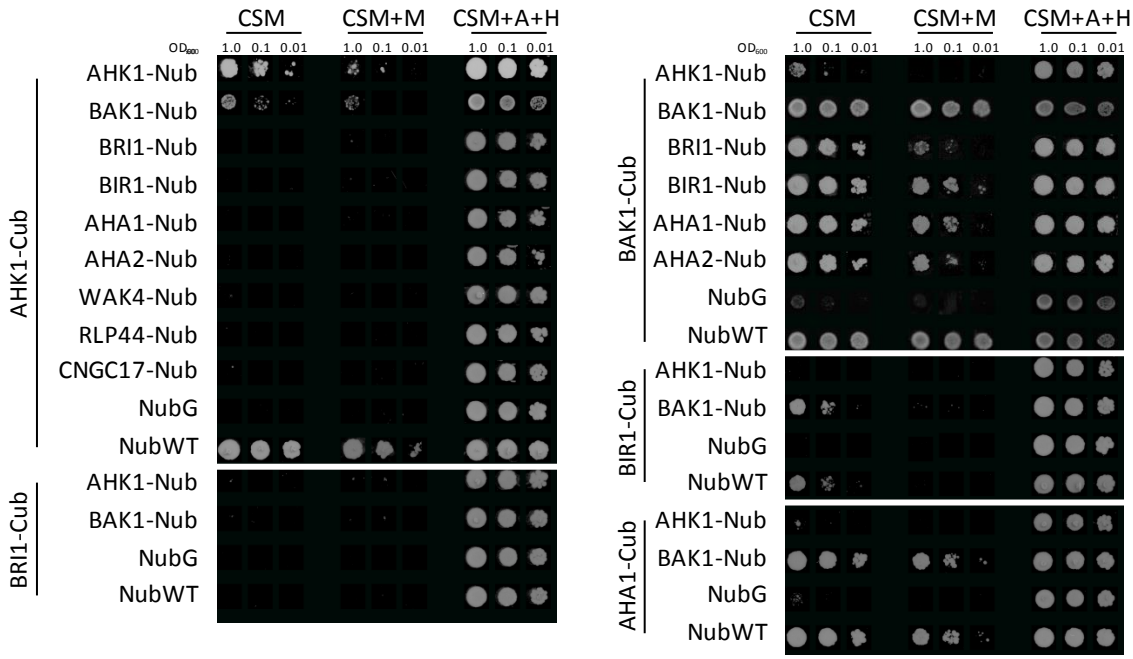



figure 4.30: Mating-based split ubiquitin assay with components of a putative supercomplex
Fusion constructs of *AHK1*, *BRI1*, *BAK1*, *BIR1* and *AHA1* with the C-terminal part of *ubiquitin* (*Cub*) were transformed into the *S. cerevisiae* strain THY.AP4, fusion constructs of *AHK1*, *BAK1*, *BRI1*, *BIR1*, *AHA1*, *AHA2*, *WAK4*, *RLP44* and *CNGC17* with the N-terminal part of *ubiquitin* (*Nub*) as well as the negative control *NubG* and the positive control *NubWT* were transformed into the *S. cerevisiae* strain THY. AP5. After the mating the interaction of the proteins was tested by dropping of the yeast on CSM minimal medium (CSM) and verified by dropping them on CSM minimal medium supplemented with 50µM Met (CSM+M) whereas CSM-Ade$^+$-His$^+$ (CSM+A+H) served as growth control. The growth was recorded after four days growth at 28°C. The detection of protein expression is shown in appendix A22.

Therefore it was very interesting that the analysis of the phosphoproteome revealed a differential phosphorylation of BRI1 at the not yet characterized phosphorylation site Ser1172 as well as differential phosphorylation of BIR1. Additionally a phosphorylated peptide originating from BIR2 was quantified as well as many phosphopeptides which belong to proteins involved in BR-signaling (table 4.7) or to LRR-RKs (appendix A33, A34, A35).

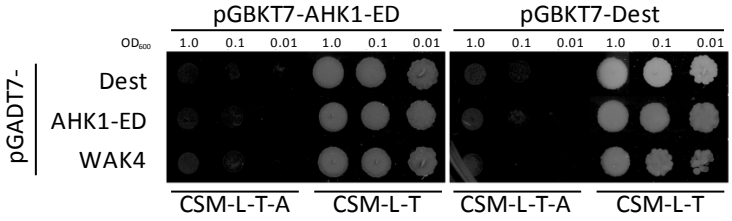



figure 4.31: AHK1-ED does not dimerize nor interact with WAK4
The reporter yeast strain pJ69-4A was co-transformed with the *BD*-fusion contruct of *AHK1-ED* (*pGBKT7-AHK1-ED*) and the *AD*-fusion constructs of *AHK1-ED* (*pGADT7-AHK1-ED*) and *WAK4* (*pGADT7-WAK4*) respectively. The empty destination-vectors (*pGBKT7-Dest*, *pGADT7-Dest*) were used as control. For the interaction test the respectively transformed yeast was plated on the auxotrophy medium CSM-Leu$^-$-Trp$^-$-Ade$^-$ (CSM-L-T-A) as well as on the growth control medium CSM-Leu$^-$-Trp$^-$ (CSM-L-T) and grown for four days at 28°C. The detection of protein expression is shown in appendix A23.

Unfortunately not all of the proteins could be cloned and tested in different interaction studies, but it could be revealed that AHK1 does not interact with BRI1, BIR1, AHA1, AHA2, WAK4, RLP44 and CNGC17 in the mbSUS whereas it interacts with BAK1 which in turn interacts with BRI1, BIR1, AHA1 and AHA2 (fig. 4.30). An additional yeast two-hybrid assay showed, that the extracellular domain of AHK1 (AHK1-ED) does not interact with the putatively extracellular kinase WAK4 neither (fig. 4.31). Additionally this assay did not reveal dimerization of AHK1-ED (fig. 4.31).

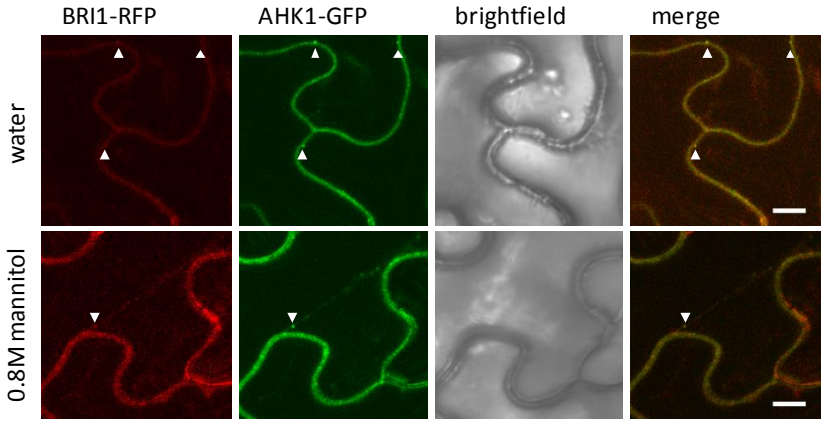



figure 4.32: Localization of BRI1-RFP and AHK1-GFP
Transient co-expression of *BRI1* tagged with a C-terminal *RFP* (*BRI1-RFP*, red) and *AHK1* tagged with a C-terminal *GFP* (*AHK1-GFP*, green) in *N. benthamiana* reveals in confokal microscopy that BRI1-RFP and AHK1-GFP co-localize at the plasma membrane and in vesicle-like structures (white arrows) in the water control as well as after treatment with 0.8M mannitol. Both proteins were expressed under the control of the *CaMV 35S-promoter*. Samples were analyzed two days after transformation. The scale is 10µm.

Although AHK1 did not interact with BRI1 in mbSUS, transiently expressed AHK1-GFP and BRI1-RFP co-localized in leaves of *Nicotiana benthamiana* (fig. 4.32) to the plasma membrane and to vesicle-like structures as they were described in 4.1.1.2. *AHK1-GFP* and *BRI1-RFP* were expressed under the control of the *CaMV 35S-promoter* but the expression was too weak for FRET-FLIM studies. For additional localization and interaction studies binary plant vectors were generated with *BIR1* fused to C-terminal *RFP* and *GFP* (*pB7RWG2-BIR1* and *pB7FWG2-BIR1*). In consideration of the new phosphorylation site of BRI1 at Ser1172 and its putative function during osmotic stress first vector constructs were generated which encode *BRI1* with Ser1172 mutated to Ala and Glu (*pDONR207-BRI1-S1172A* and *pDONR207-BRI1-S1172E*).

BRs are known to be involved in several developmental processes like for example flowering time (Li *et al.*, 2010). In one flowering time experiment it was revealed that *ahk1-3* and *ahk1-4* do not have an altered flowering time in comparison to the wildtype neither in long (fig. 4.33 B) nor short day conditions (fig. 4.33 C). The double mutant *bri1-5 ahk1-3* which was obtained by crossing had an increased number of rosette leaves at the time point of flowering (fig. 4.33 A) and flowered later than the wildtype and *ahk1-3* in long day conditions (fig. 4.33 B) whereas it exhibited the same phenotype as the *bri1-5* single mutant (fig. 4.33 A, B). In short day conditions *bri1-5 ahk1-3* flowered later than the wildtype and *ahk1-3* but earlier than *bri1-5* (fig. 4.33 C). In long day conditions *ahk1-3*/*35S::AHK1-GFP* showed a slightly earlier flowering than the wildtype, *ahk1-3* and *ahk1-4* (fig. 4.33 B).

According to Zhou *et al.* (2013) BRs are also involved in the growth of etiolated hypocotyls. Therefore three day old etiolated seedlings were analyzed upon their hypocotyl and root length.

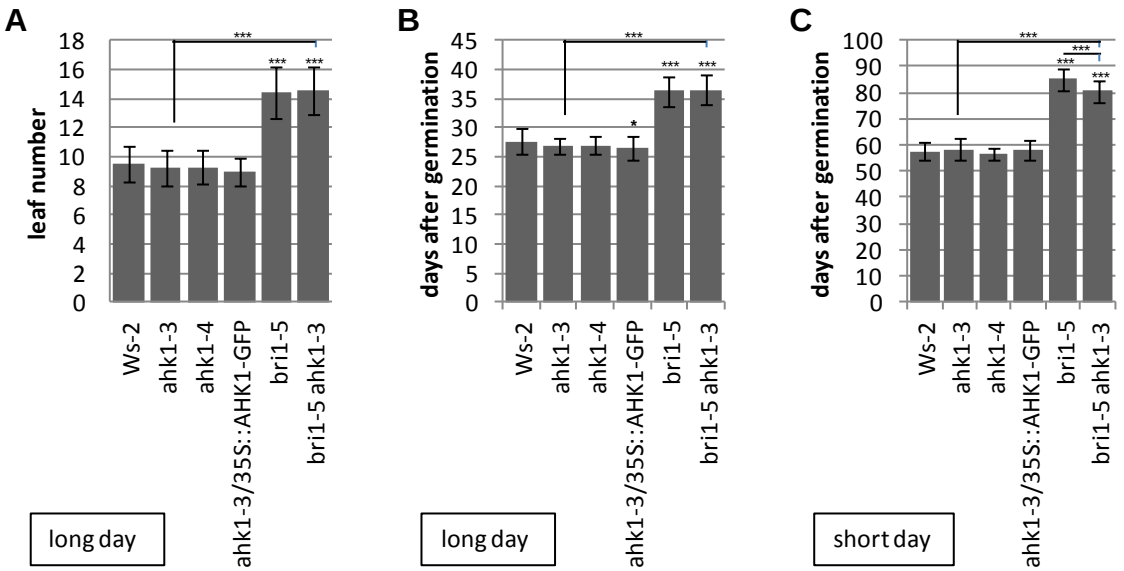



figure 4.33: Flowering time in long and short day conditions
(A) Rosette leaf number of the respective plant lines when transition from vegetative to reproductive growth takes place in long day conditions. (B) Number of days after germination when bolting of the respective plant lines starts in long day conditions. (C) Number of days after germination when bolting of the respective plant lines starts in short day conditions. Shown are mean values and standard deviations of one experiment with at least 23 plants per line and condition. Statistical significance was calculated using student's t-test. Stars above bars indicate the statistical significance of difference to the wildtype, stars above brackets give the significance of difference between the two indicated lines. *p<0.05; ***p<0.001

In four replicates with at least 32 seedlings per line and treatment *ahk1-3* and *ahk1-4* revealed decreased length of hypocotyls (fig. 4.34 A) and roots (fig. 4.34 B) in comparison to the wildtype at control conditions as well as upon treatment with 100nM brassinolide (BL) or 100nM of the inhibitor of brassinolide biosynthesis propiconazole (Pcz). *ahk1-3/35S::AHK1-GFP* showed longer hypocotyls at control conditions and upon treatment with 100nM BL. No difference in comparison to the wildtype could be observed for the hypocotyl length of *ahk1-3/35S::AHK1-GFP* after growth on 100nM Pcz as well as for the root length after growth on control conditions, 100nM BL and 100nM Pcz. *bri1-5* and *bri1-5 ahk1-3* revealed a decreased root length at all growth conditions and a decreased hypocotyl length at control conditions as well as after growth on 100nM Pcz. Supplementation with 100nM BL led to a wildtype-like hypocotyl length in *bri1-5* and *bri1-5 ahk1-3* and to an increased hypocotyl length in *bak1-1* and *bak1-1 ahk1-3* in comparison to the wildtype. In general *bak1-1* shows a shorter hypocotyl and root length than *bak1-1 ahk1-3* except for the root length at control conditions.

To verify that this effect did not derive from altered germination an additional germination assay was executed in which the germination time (fig. 4.35 A, B) was investigated in addition to the germination rate (fig. 4.35 C). At control conditions as well as on 0.3M mannitol the germination time was not altered in comparison to the wildtype for *ahk1-3*, *ahk1-4*, *ahk1-3/35S::AHK1-GFP* and *bak1-1 ahk1-3* whereas *bri1-5*, *bri1-5 ahk1-3* and *bak1-1* needed approximately half a day longer. For the germination rate no difference could be revealed neither at control conditions nor on mannitol-supplemented media for *ahk1-3*, *ahk1-4*, *ahk1-3/35S::AHK1-GFP*, *bri1*-5, *bri1-5 ahk1-3*, *bak1-1 and bak1-1 ahk1-3* except

for *bri1-5* which showed a reduced germination rate on media supplemented with 0.3M mannitol (fig4.35 C). A germination assay n BL and Pcz remains to be conveyed.

In addition to the investigation of hypocotyl and root length of three day old etiolated seedlings in the Ws-2 ecotype the experiment was repeated in the Col-0 ecotype.

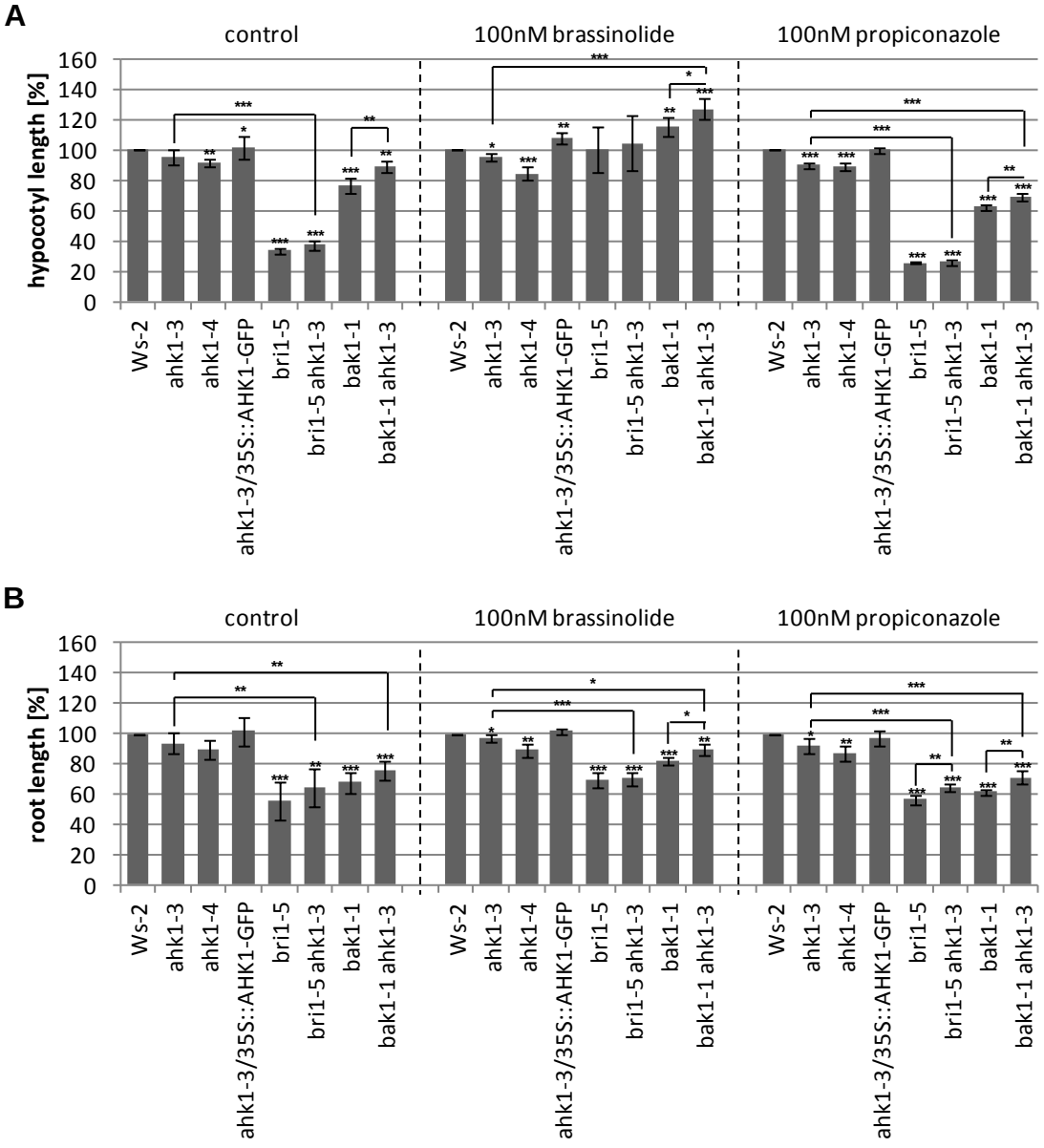



figure 4.34: Hypocotyl and root length of etiolated *ahk1* knock down seedlings of the Ws-2 ecotype after growth on brassinolide and propiconazole

Hypocotyl (A) and root (B) length of seedlings which grew in the dark for three days after 2h light induction of germination on control media and media which were supplemented with brassinolide and the brassinolide biosynthesis inhibitor propiconazole. The different treatments are separated by dashed lines.The hypocotyl and root length has been normalized to the respective wildtype. Data were averaged between four replicates with at least 32 seedlings per line and treatment. Shown are mean values and standard deviations. Student‘s ttest was used to calculate statistical significance. Stars above bars reveal statistical significance of difference in comparison to the wildtype. Stars above brackets show the statistical significance of difference between the two indicated lines. *p<0.05; **p<0.01; ***p<0.001

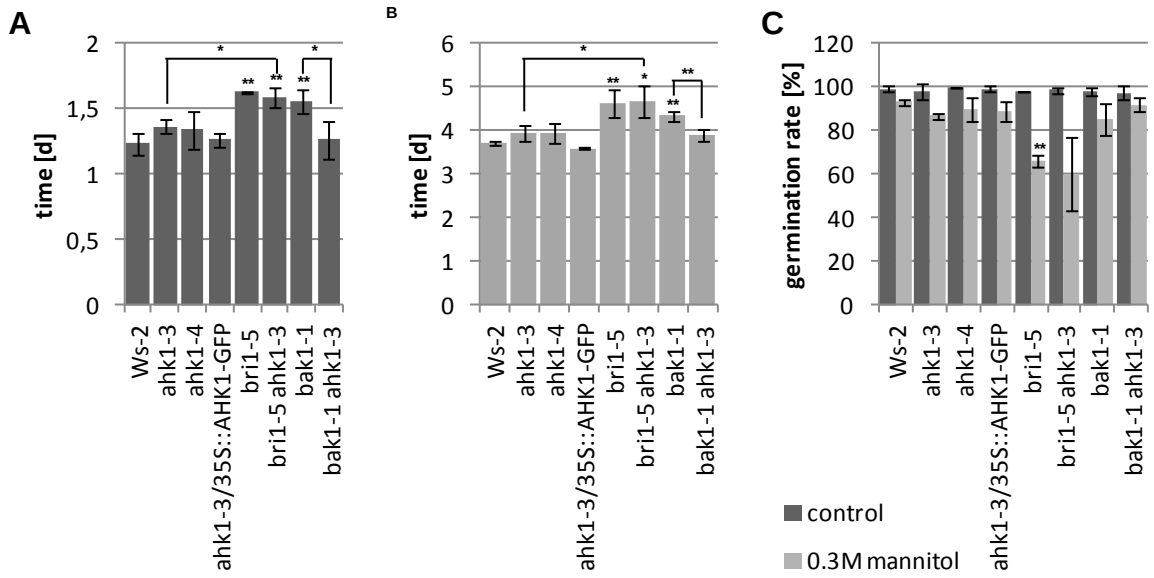



figure 4.35: Germination of *ahk1* knock down alleles
Germination time of *ahk1* knock down alleles, wildtype and controls on half strength MS salts (A) and media supplemented with 0.3M mannitol (B) after four days of stratification. (C) Germination rate of *ahk1* knock down alleles, wildtype and controls on half strength MS salts (dark grey) and media supplemented with 0.3M mannitol (light grey) after six days of constant light. Mean values and standard deviation of three replicates with 50 seeds per line and condition. Student‘s t-test was used to determine the statistical significance. * 0.01<p<0.05; ** 0.001<p<0.01.

In the Col-0 ecotype the hypocotyl length of the *ahk1* knock down alleles *ahk1-5* and *ahk1-6* was the same like in the wildtype at control conditions whereas the AHK1 overexpressor (AHK1 ox) revealed a decreased hypocotyl length. After growth on 100nM BL *ahk1-6* showed an increased hypocotyl length. A decreased hypocotyl length could be revealed for AHK1 ox on 100nM BL and for *ahk1-5* and AHK1 oc on 100nM Pcz (fig4.36 A). The root length of *ahk1-6* in comparison to the wildtype is increased at control conditions and after growth on 100nM BL. AHK1 ox showed a decreased root length in comparison to the wildtype in all conditions (fig. 4.36 B). The mutants *aha1-6* and *aha2-4* as well as *bri1-301* and *bak1-3* showed shorter length of hypocotyls and roots at control conditions and after growth on 100nM Pcz except for the root length of *aha1-6* at control conditions. BL-supplementation led to a wildtype-like length of hypocotyls and roots of *aha1-6* and *aha2-4*. Furthermore, BL-supplementation caused wildtype-like root length of *bri1-301* and longer hypocotyls in comparison to the wildtype. This could also be observed for the hypocotyl length of *bak1-3* whereas the root length stays shorter in comparison to the wildtype. The so far not characterized putative gain-of-function or knock down mutant of CNGC7 (*cngc7*, SALK_019117.56.00.x) revealed shorter hypocotyl and root length after growth on control conditions, BL and Pcz (fig. 4.36). A germination assay for the Col-0 set remains to be added.

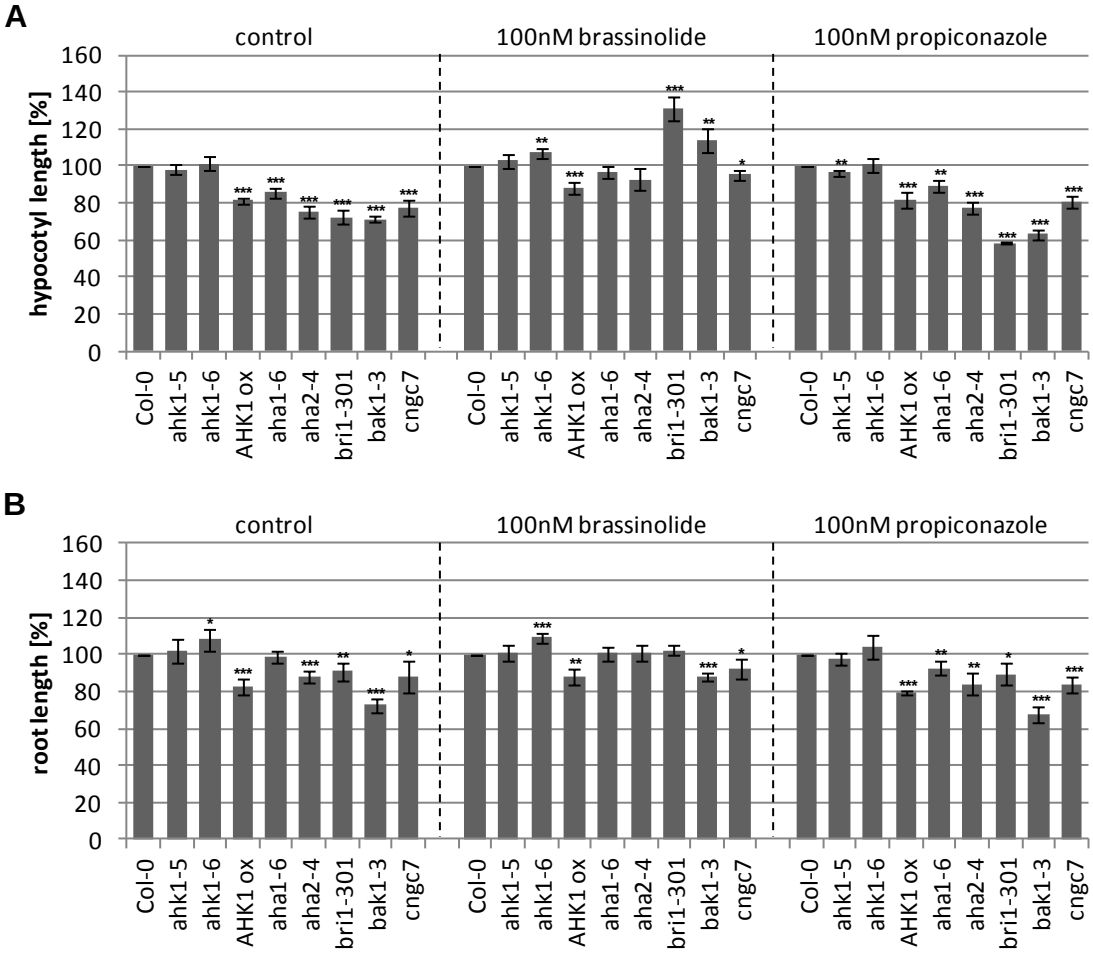



figure 4.36: Hypocotyl and root length of etiolated *ahk1* knock down seedlings in the Col-0 ecotype after growth on brassinolide and propiconazole
Hypocotyl (A) and root (B) length of seedlings which grew in the dark for three days after 2h light induction of germination on control media and media which were supplemented with brassinolide and the brassinolide biosynthesis inhibitor propiconazole. The different treatments are separated by dashed lines.The hypocotyl and root length has been normalized to the respective wildtype. Data were averaged between four replicates with at least 32 seedlings per line and treatment. Shown are mean values and standard deviations. Student‘s ttest was used to calculate statistical significance. Stars above bars reveal statistical significance of difference in comparison to the wildtype. *p<0.05; **p<0.01; ***p<0.001

The interaction studies and the length of hypocotyls and roots of the different mutants in the Ws-2 ecotype indicate a physiological relationship of AHK1 and BAK1. This arose the question whether these mutants also reveal a different response to pathogens. Therefore a pathogen assay with the necrotrophic fungus *Alternaria brassicicola* was executed by Jens Riexinger within cooperation with Birgit Kemmerling (plant biochemistry, ZMBP, University of Tuebingen, D) (Mosher *et al.*, 2013). Here the same effect could be observed like in the root growth assay. Dependent on factors which have so far not been identified, the different *Arabidopsis thaliana* mutants show opposing effects (fig. 4.37). In a first experiment with at least 18 leaves per plant line *ahk1-4* and *ahk1-3/35S::AHK1-GFP* showed a decreased disease index in comparison to the wildtype seven, ten, and fourteen days after inoculation with A*lternaria brassicicola* spores whereas *bri1-5* and *bri1-5 ahk1-3* revealed an increased disease

index. No difference to the wildtype was observed for *ahk1-3*, *bak1-1* and *bak1-1 ahk1-3* except for *bak1-1 ahk1-3* fourteen days after inoculation (fig. 4.37 B). In a second experiment an increased disease index in comparison to the wildtype was observed seven days after inoculation for *bri1-5* and for *bak1-1* seven and ten days after inoculation (fig. 4.37 C). Trypan blue staining remains to be conveyed.

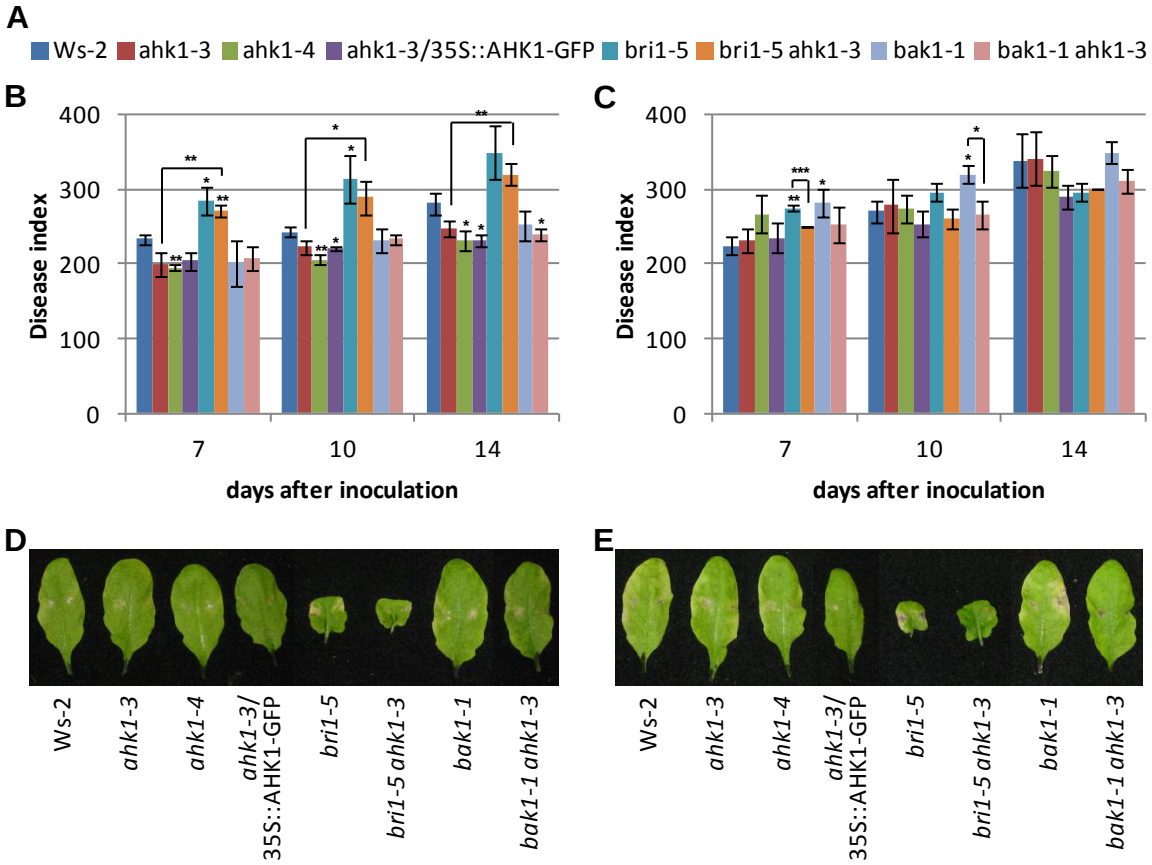



figure 4.36: Susceptibility of *ahk1* mutants to *Alternaria brassicicola*
Disease symptoms were monitored 7, 10 and 13 days after inoculation of 5 week old plants of the indicated genotypes with $10^6$ *Alternaria brassicicola* spores per ml (B, C). (A) gives the color code for (B) and (C). Shown are mean values and standard deviations of experiment 1 (B) and experiment 2 with at least 18 analyzed leaves per line (C). Student's t-test was used to calculate the significance of difference, which is indicated by stars above the bars for the significance of difference in comparison to the wildtype and above brackets for the highlighted lines. *p<0.05; **p<0.01; ***p<0.001. Pictures of representative leaves were taken thirteen days after inoculation for experiment 1 (D) and experiment 2 (E).

## 4.2 Phosphoproteomic study of short-term kinetin treatment of Col-0 and *ahk2 ahk3*

Dautel *et al.*, The Sensor Histidine Kinases AHK2 and AHK3 Proceed into Multiple Serine/Threonin/Tyrosine Phosphorylation Pathways in *Arabidopsis thaliana*, Molecular Plant (2015) is attached in appendix A31. My contribution to this paper is described in appendix A32.

# 5 DISCUSSION

In previous studies it has been suggested that AHK1 perceives osmotic stress as a mechano-sensitive receptor kinase and acts as a positive regulator of osmotic stress signaling (Urao *et al.*, 1999; Reiser *et al.*, 2003; Tran *et al.*, 2007; Wohlbach *et al.*2008). This has been contradicted by Kumar *et al.* (2013). Therefore, continuative experiments have been executed to gain insight into AHK1-dependent signal-perception and signal-transduction.

The comparison of the phosphoproteome of the *ahk1* knock down line *ahk1-3* and the wildtype Ws-2 after mock and osmotic stress treatment which was applied with the use of 0.3M mannitol revealed a general difference in the phosphoproteome of *ahk1-3* and Ws-2 but also a mannitol-dependent difference indicating that AHK1 might indeed be involved in the response to osmotic stress but also in several developmental and adaptive processes. In this as well as in previous studies, experiments which tested the influence of AHK1 on the response to osmotic stress did not reveal a constant phenotype of *ahk1* knock down lines. For example Wohlbach *et al.* (2008) and Kumar *et al.* (2013) published contradictory results of root growth assays in which they used different ecotypes and different growth conditions with, amongst others, different temperatures to test *ahk1* knock down lines. A repetition of this assay in this study with the previously analyzed three ecotypes revealed that the differences in root growth might not just depend on osmotic stress but also on temperature and light (fig. 4.7 and fig. 4.9). This is suggested by the circumstance that the plant growth chamber in which the root growth assays were executed had problems in summer to reduce the temperature to the set temperature and is confirmed by the fact, that etiolated seedlings of *ahk1* knock down alleles showed a decreased hypocotyl and root length at least in the Nos-0 and Ws-2 ecotype in comparison to the respective wildtype (fig. 4.15) at 20°C but a wildtype-like length at 28°C (fig. 4.17). This indicates that at elevated temperatures the elongation is more increased in *ahk1* knock down lines than in the wildtype and that AHK1 in fact contributes to temperature-dependent growth. Furthermore, this indicates a negative effect of AHK1 on temperature-induced hypocotyl elongation. Accordingly, elevated temperatures led to increased elongation growth in dark-grown *ahk1* knock down seedlings.

The phenotype of shorter hypocotyls and roots in etiolated *ahk1* knock down seedlings of the Ws-2 ecotype was lost to a wildtype-like hypocotyl and root length after growth at osmotic stress conditions which were applied with the use of the osmotically active substance mannitol (fig. 4.16; Osakabe *et al.* 2013). As osmotic stress is known to inhibit root elongation (Verslues *et al.*, 2006, Golldack *et al.*, 2014), the fact, that the absence of AHK1 leads to a wildtype-like hypocotyl and root length in etiolated seedlings after growth on mannitol-supplemented media and therefore to an increase in elongation growth, indicates a positive effect of AHK1 on osmotic stress regulation which has already been suggested by Tran *et al.* (2007) and Wohlbach *et al.* (2008) but contradicted by Kumar *et al.* (2013).

These observations lead to the question of the molecular mechanisms which cause the phenotype of etiolated *ahk1* knock down seedlings under control conditions but also in reaction to different treatments. In figure 5.1 a part of the plantal signal transduction network is shown which might be

involved in AHK1-dependent signaling. It comprises information about previously investigated protein interactions and phosphorylation events as well as the contribution of some phosphopeptides which were quantified in the phosphoproteome of *ahk1-3* and the wildtype Ws-2 as well as the minor contribution of phosphopeptides which were quantified in the phosphoproteome of *ahk2 ahk3* and Col-0 seedlings. With the help of this figure possible explanations can be found for the phenotype of etiolated *ahk1* knock down seedlings.

## 5.1 Increased temperature-induced hypocotyl elongation of etiolated *ahk1* knock down seedlings might depend on HY5-levels and phyD signaling

In previous studies it has been shown that warmth induces hypocotyl elongation exclusively in light-grown seedlings (Gray *et al.*, 1998; Stavang *et al.*, 2009; Oh *et al.*, 2012; Delker *et al.*, 2014). In contrast to these findings in Col-0, this study shows, that Ws-2 revealed the temperature-induced hypocotyl elongation not just in light-grown seedlings but also in etiolated seedlings (Gray *et al.*, 1998). Furthermore, *ahk1* knock down alleles show shorter hypocotyls and roots in the Ws-2 ecotype but wildtype-like hypocotyl and root length in Col-0 (fig. 4.15). Ws-2 is homozygous for a 14bp deletion in its *PHYD* gene causing the lack of phyD (Aukerman *et al.*, 1997). phyD is known to be involved especially in mediating hypocotyl elongation (Aukerman *et al.*, 1997). This suggests that the difference of the hypocotyl and root length phenotype of etiolated *ahk1* knock down alleles in the Ws-2 and Col-0 ecotype as well as the temperature-induced hypocotyl elongation in etiolated seedlings of the Ws-2 ecotype might in part depend on so far uncharacterized regulatory mechanisms of phyD. According to the already described temperature-induced hypocotyl elongation which basically depends on decreased levels of the transcription factor HY5 which in turn causes altered PIF levels and auxin responses, in etiolated seedlings this might comprise either enhanced degradation of HY5 as HY5 levels are generally low in darkness, increased inactivation of HY5 by phosphorylation at Ser36 in its COP1 binding domain, increased expression of *PIF4* which is repressed by HY5 or stabilization of PIF4 through protein-interaction for instance with BZR1 or post-transcriptional modifications (Gray *et al.*, 1998; Hardtke *et al.*, 2000; Shen *et al.*, 2005; Cheng *et al.*, 2006; Oh *et al.*, 2006; Al-Sady *et al.*, 2006; Stavang *et al.*, 2009; Stepanova *et al.*, 2011; Won *et al.*, 2011; Oh *et al.*, 2012; Park *et al.*, 2012; Toledo-Ortiz *et al.*, 2014; Delker *et al.*, 2014). The phosphoproteome of light-grown *ahk1-3* and Ws-2 seedlings revealed an AHK1-dependent differential phosphorylation of COP1 (AT2G32950) and CUL4 (AT5G46210) which both contribute to the DET1-COP1-HY5 pathway (Delker *et al.*, 2014; Lau and Deng, 2012; Nixdorf and Hoecker, 2010; Hua and Vierstra, 2011). This indicates that an alteration in HY5 degradation might occur in light but might also be influenced in darkness through AHK1-dependent post-translational modifications of other proteins.

In light-grown seedlings the phosphoproteome of *ahk1*-3 and Ws-2 did not reveal changes in the phosphorylation of HY5 (AT5G11260) itself, neither after mock nor after mannitol treatment.

Still, the phosphorylation of HY5 in darkness is conveyed by a phytochrome dependent kinase, which is putatively a casein kinase 2 (Hardtke *et al.*, 2000). So possibly the phosphorylation of HY5 is not altered in light but in darkness in a phytochrome dependent manner.

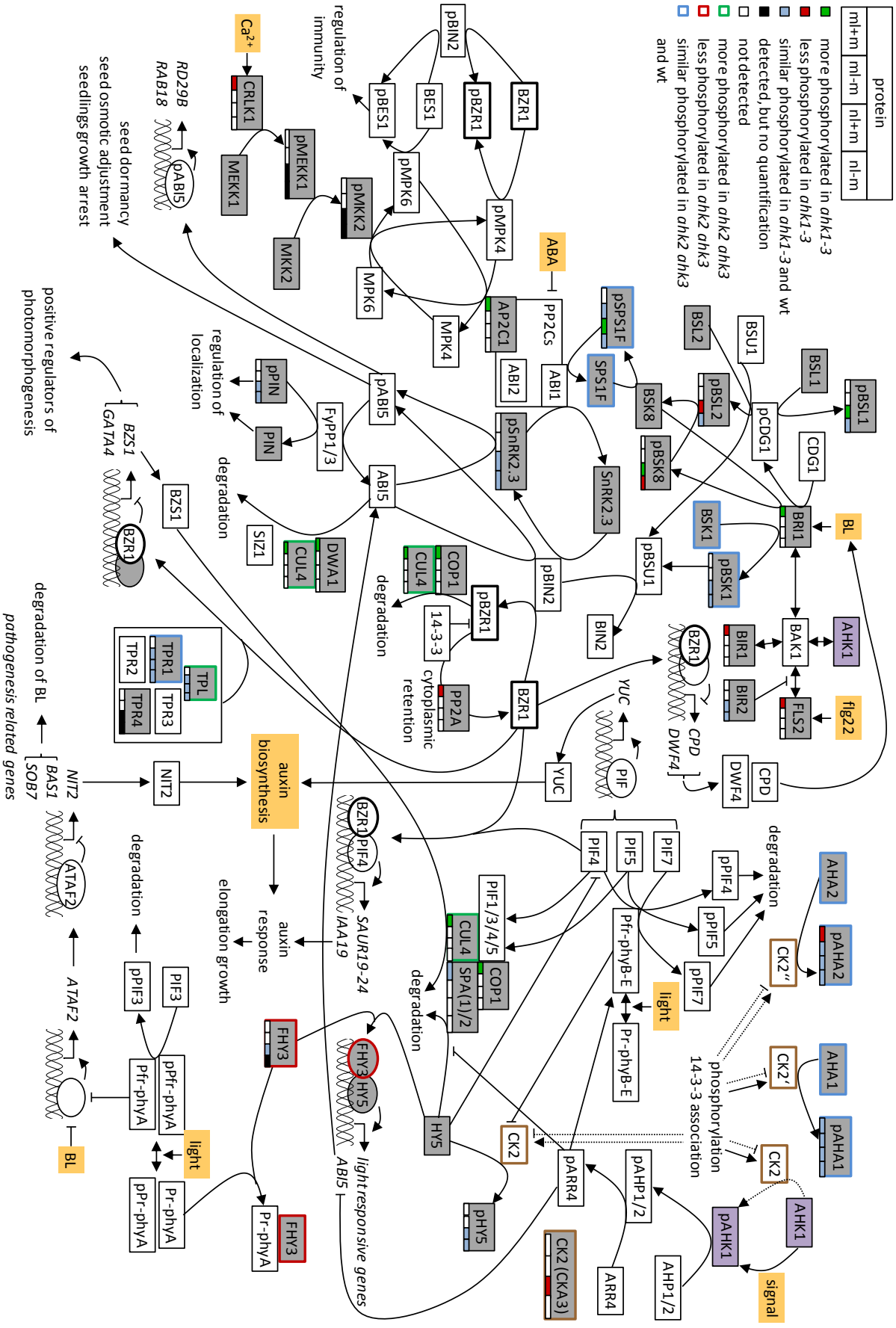

protein
nl-m
nl+m
ml-m
ml+m
more phosphorylated in ahk1-3
less phosphorylated in ahk1-3
similar phosphorylated in ahk1-3 and wt
detected, but no quantification
not detected
more phosphorylated in ahk2 ahk3
less phosphorylated in ahk2 ahk3
similar phosphorylated in ahk2 ahk3 and wt
BL
flg22
ABA
Ca2+
light
signal
phosphorylation
14-3-3 association
auxin biosynthesis
auxin response
elongation growth
degradation
cytoplasmic retention
regulation of immunity
seed dormancy
seed osmotic adjustment
seedlings growth arrest
regulation of localization
positive regulators of photomorphogenesis
degradation of BL
pathogenesis related genes
BRI1
BAK1
BIR1
BIR2
FLS2
AHK1
BSK1
pBSK1
BSK8
pBSK8
BSL1
pBSL1
BSL2
pBSL2
BSU1
pBSU1
CDG1
pCDG1
BIN2
SPS1F
pSPS1F
PP2Cs
ABI1
ABI2
AP2C1
SnRK2.3
pSnRK2.3
ABI5
pABI5
FyPP1/3
pPIN
PIN
SIZ1
CUL4
DWA1
COP1
14-3-3
pBZR1
BZR1
PP2A
BZS1
TPL
TPR1
TPR2
TPR3
TPR4
YUC
PIF
CPD
DWF4
CPD DWF4
NIT2
NIT3
BAS1
SOB7
ATAF2
RD29B
RAB18
CRLK1
MEKK1
pMEKK1
MKK2
pMKK2
MPK6
pMPK6
MPK4
pMPK4
BES1
pBES1
pBIN2
BZR1
PIF4
PIF5
PIF7
pPIF4
pPIF5
pPIF7
Pfr-phyB-E
Pr-phyB-E
PIF1/3/4/5
SPA(1)/2
SAUR19-24
IAA19
ABI5
light responsive genes
HY5
pHY5
FHY3
pPIF3
PIF3
pPfr-phyA
Pfr-phyA
Pr-phyA
pPr-phyA
ATAF2
BL
AHA2
pAHA2
AHA1
pAHA1
CK2''
CK2'
CK2
pAHK1
AHK1
AHP1/2
pAHP1/2
ARR4
pARR4
CK2 (CKA3)

figure 5.1: (previous side) Phosphorylation network with proteins of which phosphopeptides were quantified in the phosphoproteome of ahk1-*3* and the wildtype Ws-2.
Proteins of which phosphopeptides were identified and quantified in the phosphoproteome of *ahk1-3* and the wildtype Ws-2 are highlighted in grey and listed with their respective accession and $log_2$-values in appendix A33, A34 and A35. A „p“in front of the short cut of the protein‘s name designates the phosphorylation of this protein. The bar beyond the phosphorylated proteins which have been quantified in the phosphoproteome is divided in four boxes which describe whether the phosphopeptide was detected in the analysis of the phosphoproteome after metabolic labeling and 10min treatment with 0.3M mannitol (left), after metabolic labeling and 10min mock treatment (second from the left) or in a non-labeling experimental design after 10min treatment with 0.3M mannitol (second from the right) or mock treatment (right). According to the previously set threshold for differential phosphorylation the boxes in this bar highlight whether the protein was more phosphorylated in *ahk1-3* in comparison to the wildtype with $log_2(ahk1/wt) > 1$ (green), less phosphorylated with $log_2(ahk1/wt) < -1$ or similar phosphorylated with $-1 < log_2(ahk1/wt) < 1$ (blue). In case no $log_2(ahk1/wt)$-value could be obtained, the respective box is highlighted in black whereas it is white when no phosphopeptide was quantified. When boxes of proteins show contact this indicates physical protein interaction. BZR1 and pBZR1 respectively are outlined with a thick black frame due to the involvement in several pathways. Casein kinases are outlined in brown. Arrows designate the promotion of a process, arrows with a blunt end highlight the inhibition of a process. Rectangular arrows indicate gene expression. Dashed lines designate assumed regulatory mechanisms. AHK1 is highlighted in violett. Signals like light, $Ca^{2+}$, the pathogen associated molecular pattern flg22, abscissic acid (ABA), auxin and brassinolid (BL) which lead to an adaption of the plant are highlighted in yellow. Proteins of which phosphopeptides were also quantified in the phosphoproteome of *ahk2 ahk3* after 10min treatment with kinetin are outlined with a green frame for $log_2(ahk2\ ahk3/wt) > 1$, with a red frame for $log_2(ahk2\ ahk3/wt) < -1$ and with a blue frame for $-1 < log_2(ahk2\ ahk3/wt) > 1$. The references in which the shown phosphorylation and regulatory events have been described are noted in the text.

This might occur through different mechanisms. It is known, that the Pr- as well as the Pfr-form of phytochromes can occur phosphorylated (Kim *et al.*, 2005). For instance, for phyA it has been shown that the underphosphorylated Pr-form of phyA preferentially interacts with the transcription factor FHY3 which prohibits its translocation to the nucleus and therefore represses its effect on gene expression (Seo *et al.*; 2004; Saijo *et al.*, 2008). Such a mechanism might also exist for phyB to phyE. In addition, phyB to phyE are known to form heterodimers (Sharrock and Clack, 2004). This might influence the specificity of interaction with different target proteins. Therefore the lack of phyD in the Ws-2 ecotype might lead to a gap in heterodimerization which might alter specific responses through varied protein-interactions which in turn causes in the case of temperature-induced hypocotyl elongation, lower HY5 levels, increased PIF4 levels or influences other pathways which induce elongation growth upon elevated temperatures (Gray *et al.*, 1998; Sharrock and Clack, 2004; Delker *et al.*, 2014).
According to the negative effect of AHK1 on temperature-induced hypocotyl elongation in etiolated seedlings of the Ws-2 ecotype, components of this pathway might be phosphorylated in an AHK1-dependent manner. This might be achieved through an AHK1-dependent phosphorelay which activates type-B response regulators which can directly regulate gene expression possibily including the expression of *PIF4* (Urao *et al.*, 2000; Dortay *et al.*, 2006; Mira-Rodado *et al.*, 2007; Dortay *et al.*, 2008). Furthermore AHK1-dependent type-A response regulators might regulate kinases or phosphatases which in turn regulate enzyme activity (Urao *et al.*, 2000; Sweere *et al.*, 2001; Dortay *et al.*, 2006; Mira-Rodado *et al.*, 2007; Dortay *et al.*, 2008). For instance it could be suggested that ARR4 is activated in an AHK1 dependent manner to interact with phyB or the protein phosphatase 2C family protein AT3G12620 (Sweere *et al.*, 2001; Mira-Rodado *et al.*, 2007; Dortay *et al.*, 2008). A comparable regulation of enzyme activity might in turn be involved in an altered activity of the E3 ligase comprising

COP1 and CUL4 which are shown to be differentially phosphorylated in an AHK1-dependent manner and which are known to target HY5 for degradation (Hardtke *et al.*, 2000; Hua and Vierstra, 2011; Delker *et al.*, 2014).

## 5.2 AHK1 might contribute to brassinosteroid signaling through the interaction with BAK1

The positive effect of AHK1 on osmotic stress regulation might be correlated with brassinosteroid (BR) signaling as the phosphoproteome of *ahk1-3* and the wildtype Ws-2 revealed several proteins which are known to be involved in BR signaling to be differentially phosphorylated in *ahk1-3* in comparison to the wildtype Ws-2 upon mock or osmotic stress treatment which was applied with the use of 0.3M mannitol (Tran *et al.*, 2007; Wohlbach *et al.*, 2008). Furthermore, the altered temperature-induced hypocotyl elongation suggests a contribution of PIF4 and BZR1 to AHK1-dependent elongation growth whereas BZR1 is a component of BR signaling (Oh *et al.*, 2012). In addition it has previously been shown by Katharina Caesar that AHK1 interacts with BAK1 (unpublished data). BAK1 in turn interacts with many other proteins including the brassinosteroid receptor BRI1, the receptor of the pathogen-associated molecular pattern (PAMP) flg22 FLS2, $H^+$-ATPases and cyclic nucleotide gated channels (CNGCs) (Li *et al.*, 2002b; Sun *et al.*, 2013; Ladwig *et al.*, 2015; fig. 4.30). Knock down mutants of BRI1, BAK1, AHK1, AHA1 and AHA2 as well as a putative gain-of-function or knock down mutant of CNGC7 (*cngc7*; SALK_019117.56.00.x) reveal decreased elongation growth of hypocotyls and roots in etiolated seedlings in comparison to the respective wildtype (fig. 4.34 and fig. 4.36) indicating a contribution of all these proteins in the regulation of skotomorphogenesis. Thereby, the double mutant *bri1-5 ahk1-3* revealed the *bri1-5* phenotype indicating BRI1 acting epistatic to AHK1 (fig. 4.34). Etiolated seedlings of the double mutant *bak1-1 ahk1-3* revealed *ahk1-3*-like hypocotyl length but *bak1-1*-like root length indicating a tissue-specific epistasis of AHK1 and BAK1 under control conditions. The generation and analysis of a *bri1-5 bak1-1 ahk1-3* triple mutant remains to be conveyed. Still, it can be assumed, that AHK1 influences BR signaling. This might occur through different ways. One way might be the AHK1-dependent change of abundance of BZR1 interaction partners like it was suggested for PIF4 during elevated temperatures (Oh *et al.*, 2012). Another way might be initiated by the interaction of AHK1 and BAK1. It has been shown that BAK1-mediated signaling specificity depends on the transphosphorylation pattern of BAK1 and its interacting receptor-like kinases (RLKs) (Wang *et al.*, 2014a). In addition it is assumed, that distinctive transphosphorylation patterns of BAK1 interacting RLKs can be generated when the phosphorylation status of BAK1 is altered (Wang *et al.*, 2014a). This means that the interaction of BAK1 with different RLKs or other proteins like AHK1 might vary the phosphorylation status of BAK1 and therefore the transphosphorylation pattern and activity of the RLKs including BRI1 (Wang *et al.*, 2008). This would provide an explanation for the differential phosphorylation of BRI1 at Ser1172 in light-grown 14d old *ahk1-3* seedlings in comparison to the wildtype after 10min treatment with 0.3M mannitol (Wang *et al.*, 2008). It has to be noted that this is an until now unidentified phosphorylation site of BRI1 (Wang *et al.*, 2008). This differential phosphorylation of BRI1 might subsequently lead to altered activity or interaction of BRI1 with proteins and to a difference in the phosphorylation status of these proteins. An

altered activity of BRI1 by the AHK1-dependent phosphorylation at Ser1172 can be suggested due to the phenotype which was observed in etiolated seedlings which grew on 100nM brassinolide (BL) or the BR-biosynthesis inhibitor propiconazole (Pcz) respectively (fig. 4.34). As BAK1 and BRI1 transphosphorylate upon BL-perception which initiates phosphorylation cascades and finally amongst others leads to elongation growth the disruption of BAK1 in *bak1-1* leads to a decrease of hypocotyl elongation under control conditions (Wang *et al.*, 2008; Wang *et al.*, 2012b). BL-supplementation might cause the enhanced elongation growth of hypocotyls of etiolated *bak1-1* seedlings through increased activation of BRI1. This occurs in presence of AHK1 which might mediate the phosphorylation or the loss of phosphorylation of BRI1 at Ser1172. The disruption of AHK1 in *bak1-1* might possibly lead to the change of phosphorylation at Ser1172 and might thereby cause the enhanced hypocotyl length of etiolated *bak1-1 ahk1-3* seedlings indicating a positive effect of this phosphorylation site on BRI1 activity. The AHK1-dependent phosphorylation of BRI1 at Ser1172 seems thereby to be BL-independent as etiolated *bak1-1 ahk1-3* seedlings which grew on media supplemented with 100nM Pcz revealed an increased hypocotyl length in comparison to *bak1-1* indicating that the AHK1-dependent change of BRI1 phosphorylation at Ser1172 still causes increased BRI1-activity and BR-signaling which causes increased elongation growth. In addition, this indicates that the phosphorylation of BRI1 at Ser1172 might be independent of BAK1 transphosphorylation although it can not be excluded. It remains to be elucidated whether the AHK1-dependent phosphorylation of BRI1 at Ser1172 is enhanced or reduced in etiolated seedlings.

Furthermore, the phosphorylation of BRI1 at Ser1172 might lead to a difference of BRI1 interaction with other proteins as several proteins in the BRI1-dependent phosphorylation cascade show differential phosphorylation in *ahk1-3* and Ws-2. It is known, that BRI1 phosphorylates and therefore activates the kinase CDG1 (Kim *et al.*, 2011). CDG1 unfortunately was not quantified itself in the phosphoproteome of *ahk1-3* and Ws-2 but active CDG1 is known to phosphorylate and activate the phosphatases BSU1, BSL1 and BSL2 (Kim *et al.*; 2011). The phosphatase BSL2 (AT1G08420) was identified to be less phosphorylated at Thr147 in *ahk1-3* in comparison to the wildtype after mannitol treatment and is known to dephosporylate and activate the kinase BSK8 (Wu *et al.*, 2014). BSK8 (AT5G41260) in turn was more phosphorylated in *ahk1-3* at Ser213 after mannitol treatment and less phosphorylated at this site after mock treatment whereas Ser20 did not show differential phosphorylation. Wu *et al.* (2014) showed that the phosphorylation of BSK8 at Ser213 reduces the ability of BSK8 to deactivate SPS1F (AT5G20280) through its phosphorylation at Ser152. SPS1F is involved in the regulation of sucrose metabolism and sucrose in turn is an osmotically active substance (Wu *et al.*, 2013; Osakabe *et al.*; 2013). Therefore SPS1F might play a role in osmotic adjustment. The phosphorylation of SPS1F at Ser152 was not altered in *ahk1-3* neither after mock nor mannitol treatment but increased for Ser684, Ser688 and Ser700. The role of these phosphorylation sites in protein interaction and activity of SPS1F remains to be elucidated. The deactivation of SPS1F through phosphorylation at Ser152 can be rejected by dephosphorylation by the protein phosphatase 2C (PP2C) family protein ABI1 which has not been quantified in the phosphoproteome of *ahk1-3* and Ws-2 but whose activity is known to be inhibited upon ABA signaling (Nishimura *et al.*, 2010; Finkelstein, 2013).

The differential phosphorylation of the components of this pathway suggests that the AHK1-dependent phosphorylation of BRI1 at Ser1172 might indeed play a role in the binding affinity or phosphorylation effectiveness of BRI1 towards CDG1. Thereby, the phosphorylation status of CDG1 might alter its binding specificity as CDG1 is known to phosphorylate and activate BSL1 (AT4G03080) and BSL2 whereat BSL1 revealed an increased phosphorylation and BSL2 a decreased phosphorylation in *ahk1-3* in comparison to the wildtype after mannitol treatment. The decreased phosphorylation of the phosphatase BSL2 and therefore its decreased activity might cause the increased phosphorylation of BSK8 in *ahk1-3* in comparison to the wildtype after mannitol treatment (Wu *et al.*, 2014). In addition the increased phosphorylation of BSK8 might be explained by an increased kinase activity of BRI1 directly towards BSK8 as BRI1 and BSK8 have been shown to interact as well (Sreeramulu *et al.*, 2013). Therefore it remains to be elucidated, which kinase targets the respective phosphorylation site.

## 5.3 Altered BZR1-levels might explain hypocotyl and root length phenotype of etiolated *ahk1* knock down seedlings.

Still, this does not explain the reduced hypocotyl and root length of etiolated *ahk1* knock down seedlings. An important link between light-, BR- and auxin-signaling is the transcription factor BZR1 (Oh *et al.*, 2012; Oh *et al.*, 2014; Delker *et al.*, 2014). BZR1 can be phosphorylated (pBZR1) by the phosphorylated and therefore active kinase BIN2 as well as by the kinase MPK4 (He *et al.*, 2002; Li *et al.*, 2002a; Kim *et al.*, 2009; Wang *et al.*, 2013). Upon phosphorylation, BZR1 is known to be bound by 14-3-3 proteins, translocalized from the nucleus to the cytoplasm and therefore less active as transcription factor (Gampala *et al.*, 2007). On the one hand pBZR1 is targeted for degradation through the interaction with the COP1 comprising E3-ligase which is in darkness active in the nucleus (He *et al.*, 2002; Yin *et al.*, 2002; Hua and Vierstra, 2011). On the other hand, pBZR1 can be dephosphorylated during cytoplasmic retention by protein phosphatase 2A (PP2A) family proteins which are heterotrimeric serine/threonine phosphatases which are composed of a scaffolding A subunit, a regulatory B subunit and a catalytic C subunit (Tang *et al.*, 2011; Farkas *et al.*, 2007). Subsequently BZR1 is relocated to the nucleus mediating activation and repression of gene expression (Tang *et al.*, 2011).

The analysis of the phosphoproteome of *ahk1-3* and the wildtype Ws-2 after mannitol treatment revealed several proteins to be differentially phosphorylated which are involved in the regulation of BZR1 activity. One of these proteins is the protein phosphatase 2C (PP2C) family protein AP2C1 (AT2G30020) which revealed increased phosphorylation in *ahk1-3* after mannitol treatment and which dephosphorylates and thereby inactivates MPK4 which in turn leads to decreased levels of pBZR1 (Schweighofer *et al.*, 2007; Asai *et al.*, 2002; Wang *et al.*, 2013; Kang *et al.*, 2015b). Another protein which was identified to be less phosphorylated in *ahk1-3* after mannitol treatment and which is known to directly interact with BZR1 is a protein phosphatase 2A (PP2A) regulatory B subunit (AT5G25510) which leads to dephosphorylation of cytoplasmic pBZR1 and therefore to the nuclear translocation and reactivation of BZR1 (Farkas *et al.*, 2007; Tang *et al.*, 2011). Furthermore, COP1 and CUL4 which are components of the E3-ligase which is assumed to target pBZR1 for degradation in darkness reveal differential phosphorylation in *ahk1-3* in comparison to the wildtype after mannitol treatment (Kim *et al.*,

2014). The altered phosphorylation of AP2C1, PP2A regulatory B subunit, COP1 and CUL4 might cause altered binding affinities of the COP1 E3-ligase towards pBZR1, to changes in the enzyme activity or to changes in the complex composition and therefore to altered target specificity. Unfortunately the identified phosphorylation sites of all these proteins are not yet characterized in regard to their influence on enzyme activity, complex association or BZR1 binding capability. Still, it can be assumed that these post-translational modifications which occur AHK1- and mannitol-dependent might play a role in the observed *ahk1* phenotype of decreased hypocotyl and root growth. This might work through decreased BZR1 levels or activity in etiolated seedlings of *ahk1* knock down lines of the Ws-2 ecotype during growth at control conditions which might be achieved by decreased phosphatase activity of AP2C1 through altered phosphorylation, less binding capability towards BZR1 of PP2A which might occur due to altered PP2A complex association through altered phosphorylation of the BZR1 binding PP2A regulatory B subunit as well as through increased degradation of pBZR1 by the COP1 E3 ligase. Decreased BZR1 levels or activity might in cooperation with PIF4 reduce the promotion of the expression of the auxin response genes *SAUR19* to *SAUR24*, *IAA19* and *IAA29* which leads to a decrease in auxin response and therefore weakened elongation growth (Franklin *et al.*, 2011; Oh *et al.*, 2012; Delker *et al.*, 2014). In addition, less active BZR1 might lead to slightly increased expression of positive regulators of photomorphogenesis like BZS1 and GATA4 which might also reduce the elongation growth of hypocotyls and roots (Luo *et al.*, 2010; Fan *et al.*, 2012). Furthermore less active BZR1 might also lead to slightly increased expression of *CPD* and *DWF4* (He *et al.*, 2005; Gampala *et al.*, 2007). CPD and DWF4 are in general involved in BR-biosynthesis so the upregulation of their expression by less active BZR1 provides a feedback loop, as more biosynthesis of, for instance, the brassinosteroid brassinolide (BL) leads to more BL which is perceived by BRI1 (He *et al.* 2005; Gampala *et al.*, 2007; Tang *et al.*, 2008).This would subsequently lead to the phosphorylation and activation of the kinase BSK1 which activates the phosphatase BSU1 (Tang *et al.*, 2008; Kim *et al.*, 2010; Kim *et al.*, 2010). BSU1 in turn deactivates the kinase BIN2 through dephosphorylation which leads to less phosphorylation of BZR1 and therefore to increased levels of active BZR1, which in turn reduce the expression of *CPD* and *DWF4* (Li *et al.*, 2002a; He *et al.*, 2002; Wang *et al.*, 2002; He *et al.*, 2005; Gampala *et al.* 2007; Kim *et al.*, 2009). This up- and down-regulation of *CPD* and *DWF4* expression is independent of PIF4 and might thus, be influenced by additional transcriptional regulators which are shown to interact with BZR1 and which reveal differential phosphorylation in *ahk1-3* in comparison to the wildtype like for example TPL and TPR proteins, Aux/IAAs and ARFs or by transcriptional regulators whose expression is regulated by active BZR1 (Oh *et al*, 2012; Causier *et al.*, 2012; Oh *et al.*, 2014). According to the phosphoproteome which also showed the mannitol dependence of these phosphorylation events it might be possible that upon osmotic stress or mannitol-treatment, the previously decreased BZR1 levels or activity in *ahk1* knock down mutants are upregulated which in turn leads to increased elongation growth which causes the wildtype-like hypocotyl and root length of etiolated *ahk1* knock down seedlings after growth on osmotic stress media.

Thereby it has to be noted, that the regulation of BZR1 levels and activity does not only depend on BR signaling but also on phyB and light signaling (Kim *et al.*, 2014; Sun *et al.*, 2010; Oh *et al.*, 2012).

Although it has been shown, that BRI1 acts epistatically to phyB, the absence of phyB as well as dark treatment activates BR signaling which increases BZR1 activity which in turn causes hypocotyl growth (Kim *et al.*, 2014).

As etiolated *ahk1* knock down seedlings exhibit decreased hypocotyl elongation growth, AHK1 might contribute to this pathway as positive regulator on the one hand as previously described through the interaction of AHK1 with BAK1 and the AHK1-dependent change of phosphorylation of BRI1 at Ser1172 (Wang *et al.*, 2008).

## 5.4 ARR4 might provide a link between AHK1-dependent light-, brassinosteroid- and abscissic acid-signaling

On the other the other hand AHK1 might be involved in this pathway by the change of the phosphorylation status of components of the multistep phosphorelay system. It has been shown that in the multistep phosphorelay the phosphate group is transferred from AHK1 to either AHP1 or AHP2 (Urao *et al.*,2000; Dortay *et al.*, 2006). From AHP1 and AHP2 the phosphate can be transferred to either the type-A response regulators ARR3, ARR4, ARR7 or ARR9 or to the type-B response regulator ARR1 (Urao *et al.*, 2000; Suzuki *et al.*, 2001; Dortay *et al.* 2006; Mira-Rodado *et al.*, 2007; Dortay *et al.*, 2008). In addition, the phosphate can be transferred from AHP1 to the type-B response regulator ARR10 or ARR14 and from AHP2 to the type-B response regulator ARR11 (Dortay *et al.*, 2006). The type-B response regulators might in turn regulate the expression of type-A ARRs as well as the expression of further response genes whereas the type-A response regulators might be involved in the regulation of the activity of additional enzymes (Sakai *et al.*, 2001; Hwang and Sheen, 2001; Lohrmann and Harter, 2002). For ARR4 it has already been shown that phosphorylated ARR4 (pARR4) interacts with phyB in its Pr- and Pfr-form but stabilizes phyB in the active Pfr-form in light conditions (Sweere *et al.*, 2001; Mira-Rodado *et al.*, 2007). This might lead to increased phosphorylation and consequently increased degradation of PIFs by the active phyB (Shen *et al.*, 2005; Oh *et al.*, 2006; Al-Sady *et al.*, 2006; Li *et al.*, 2012; Rolauffs *et al.*, 2012). The light-dependent increased degradation of PIFs in turn leads to a further decrease of the activity of the light-inactivated COP1 E3-ligase towards targeting of the activator of light-induced gene expression HY5 to degradation (Rolauffs *et al.*, 2012; Xu *et al.*, 2014c; Jang *et al.*, 2014; Lau and Deng, 2012). Hence, pARR4 might be able to inhibit the degradation of HY5 and to promote the expression of light-responsive genes in light conditions.

Nevertheless it has been shown that ARR4 protein levels remained below the detection limit in dark-grown seedlings and were not induced before red light stimuli (Sweere *et al.*, 2001). Furthermore, ARR4 has not been detected in roots at all whereas AHK1 in contrast is mainly expressed in roots (Sweere *et al.*, 2001; Urao *et al.*, 1999; Dinkel *et al.*, 2016).

Still, a contribution of ARR4 in AHK1-dependent regulation of osmotic stress cannot be ruled out completely as Wohlbach *et al.* (2008) suggested a role of ARR3, ARR4, ARR8 and ARR9 in osmotic stress regulation. This hypothesis depends on the finding that the disruption of ARR3, ARR4, ARR5 and ARR6 leads to an increased sensitivity to osmotic stress and that this phenotype can be repressed by the additional disruption of ARR8 and ARR9 (Wohlbach *et al.*, 2008; Salomé *et al.*, 2006). In this

regard it is interesting, that ARR4, which is present in light-grown seedlings, is also known to interact with the PP2C family protein AT3G12620 (Dortay *et al.*, 2008). Possibly, in light conditions ARR4 is involved in the AHK1-dependent phosphorelay which leads to an altered activity of the phophatase AT3G12620 and therefore to a whole set of differentially phosphorylated proteins upon AHK1 signaling. AT3G12620 was unfortunately not quantified in the phosphoproteome of *ahk1-3* and the wildtype, neither after mock nor after mannitol treatment. Thereby it has be to noted, that although AHK1 is mainly expressed in roots, at least low AHK1 expression levels could be detected in the stem and in the leaves (Winter *et al.*, 2007). In regard to the abundance of ARR4 in the shoot of light-grown plants and the possibility of an ARR4 dependent transition to Ser/Thr/Tyr phosphorylation by the regulation of a PP2C family protein, not many AHK1 receptor kinase proteins are necessary to initiate first the phosphorelay without signal enhancement and second through ARR4 the PP2C-dependent signal cascade which amplifies the input signal (Sweere *et al.* 2001; Winter *et al*, 2007).

A contribution of ARR4 in AHK1-dependent signaling is further confirmed by the fact, that phosphorylated ARR4 is known to inhibit *ABI5* expression (Wang *et al.*, 2011a). ABI5 in its unphosphorylated form is targeted for degradation by ubiquitination and by sumoylation (Lee *et al.* 2010; Miura *et al.*, 2009). The ubiquitination is conferred by an E3-ligase which comprises the in *ahk1-3* differentially phosphorylated CUL4 as scaffold protein and DWA1 as substrate recognition subunit for ABI5 (Lee *et al.*, 2010; Hua and Vierstra, 2011). The sumoylation of ABI5 is conferred by SIZ1 (Miura *et al.*, 2009). Phosphorylation of ABI5 increases its DNA binding affinity and is conveyed by the phosphorylated and thereby active kinase BIN2 (pBIN2) and SnRK2.3 (Lynch *et al.*, 2012; Yuan *et al.*, 2013; Hu and Yu, 2014). Upon this activation ABI5 is involved in seed dormancy and processes of the early seedling development like seed osmotic adjustment and seedlings growth arrest (Carles *et al.*, 2002; Hirayama *et al.*, 2007). Furthermore, phosphoryated ABI5 (pABI5) promotes, amongst others, the expression of *RD29B* and *RAB18* (Carles *et al.*, 2002). Tran *et al.* (2007) could show that the expression of these two genes is decreased in *ahk1* knock down lines in comparison to the wildtype in unstressed plants as well as after 2.5h of dehydration stress. This indicates that the abundance of pABI5 is decreased in *ahk1* knock down lines. The abundance of pABI5 can be changed by altered activities of phosphorylating enzymes, dephosphorylating enzymes or through degradation. The phosphoproteome of *ahk1-3* and the wildtype did not reveal a change in the phosphorylation of the ABI45 phosphorylating enzymes SnRK2.3 (AT5G66880) or BIN2 (AT4G18710), neither after mock nor mannitol treatment, indicating that their activity is not altered. The dephosphorylation of pABI5, seems not to be influenced in *ahk1-3* neither, as this is conveyed by FyPP1 and FyPP3, which are also dephosphorylating PIN proteins (Dai *et al.*, 2012; Dai *et al.*, 2013; Yuan *et al.*, 2013). As PIN3 and PIN7 did not reveal differential phosphorylation in *ahk1-3* in comparison to the wildtype neither after mock nor mannitol treatment, the activity of FyPPs might be similar in *ahk1-3* and the wildtype (Dai *et al.*, 2012). In fact, differential phosphorylation in *ahk1-3* and the wildtype was observed for CUL4 and DWA1 which are components of an E3-ligase (Hua and Vierstra, 2011). This indicates that the decrease of pABI5 which causes the decreased expression of *RD29B* and *RAB18* depends on increased targeting for degradation of ABI5 in *ahk1* knock down lines.

Beside post-translational modifications, a decrease in abundance of pABI5 could be caused by increased inhibition of *ABI5* expression might occur. This would imply increased levels of pARR4 which inhibit *ABI5* expression. *ARR4* expression is shown to be upregulated either by light or by cytokinin and ARR4 is shown to interact with and might therefore be phosphorylated by AHP1, AHP2, AHP3 and AHP5 (D'Agostino *et al.*, 2000; Sweere *et al.*, 2001; Urao *et al.*, 2000; Dortay *et al.*, 2006; Mira-Rodado *et al.*, 2007; Dortay *et al.*, 2008). These phosphotransferproteins are known to act redundantly with more than one histidine kinase (Dortay *et al.* 2006; Dortay *et al.*, 2008). Therefore, the phosphorelay which leads to a phosphorylation of ARR4 might be initiated by the cytokinin receptors AHK2, AHK3 and AHK4 or by CKI1 which is shown to be able to form heterodimers with AHK1 (fig. 4.13) or by AHK5 (Urao *et al.*, 2000; Dortay *et al.*, 2006; Mira-Rodado *et al.*, 2007; Dortay *et al.*, 2008; Pekárová *et al.*, 2011; Bauer *et al.*, 2013). The phosphoproteome of *ahk1-3* and the wildtype Ws-2 revealed differential phosphorylation of AHK4 after mannitol treatment at phosphorylation sites which are located closely to the receiver domain which is important for the interaction with AHPs to convey the phosphorelay (Dinkel *et al.*, 2016). Probably this decrease in phosphorylation leads to altered binding specificities of AHPs and favoured phosphorelay components. Alternatively such phosphorylations might lead to conformational changes and therefore to an altered kinase activity. This indicates an AHK1-dependent regulation of cytokinin signaling. This hypothesis is confirmed by the phenotype of etiolated *ahk1* knock down seedlings as they reveal shorter roots and hypocotyls. This has previously been discussed in context with BR signaling. In addition to that it is known that the application of exogenous cytokinin leads first to shorter hyocotyls in etiolated wildtype seedlings and later on to open cotyledons and the development of true leaves in darkness (Chory *et al.*, 1994). The shorter hypocotyls and roots of etiolated *ahk1* knock down seedlings after growth on media without supplements might thus be an indication for increased endogenous cytokinin levels. In this regard it might be interesting, that the analysis of the phosphoproteome of *ahk1-3* and the wildtype after mannitol treatment revealed more phosphorylation of CYP735A1 (AT5G3845) and less phosphorylation of LOG7 (AT5G06300) in *ahk1-3* which are both involved in the biosynthesis of cytokinin and which implies that they are AHK1- and mannitol-dependent differentially regulated (Kieber and Schaller 2014). This, in combination with the loss of the shorter hypocotyl and root phenotype of etiolated *ahk1* knock down seedlings after growth on mannitol-supplemented media indicates that etiolated *ahk1* knock down seedlings may have an increased endogenous cytokinin level which might be drastically decreased upon osmotic stress as etiolated *ahk1* knock down seedlings which were grown on mannitol supplemented media to apply osmotic stress revealed wildtype-like hypocotyl length (fig. 4.16). The assumption that osmotic stress leads to a decrease in endogenous cytokinin levels indicates a negative effect of cytokinin to osmotic stress adaption. This fits to the hypothesis of Tran *et al.* (2007) who proposed a negative effect of AHK2, AHK3 and AHK4 which are known as cytokinin receptors on osmotic stress signaling. AHK1 might thereby act as negative regulator of cytokinin signaling. This might be proposed due to the finding that the absence of AHK1 decreases hypocotyl and root length of etiolated seedlings (fig. 4.15) but that treatment with exogenous cytokinin, which is known to cause decreased hypocotyl elongation (Chory *et al.*, 1994),

leads to a higher decrease of hypocotyl length in etiolated seedlings in the absence of AHK1 (fig. 4.19).

The absence of the AHK1-dependent negative regulation of cytokinin signaling in etiolated *ahk1* knock down seedlings which might cause increased cytokinin levels might subsequently lead to an increase in pARR4 levels which inhibit *ABI5* expression and might therefore lead to decreased pABI5 levels which might result in the previously detected decrease of *RD29B* and *RAB18* expression (D'Agostino *et al.*, 2000; Carles *et al.*, 2002; Hirayama *et al.*, 2007; Tran *et al.*, 2007; Wang *et al.*, 2011a). Besides, the differential regulation of ABI5 in *ahk1* knock down lines does not just provide an explanation for the decreased expression of *RD29B* and *RAB18* but also for the altered ABA responses of *ahk1* knock down lines which have previously been described and which are linked to ABI5 signaling (Tran *et al.*, 2007; Wohlbach *et al.*, 2008; Kumar *et al.*, 2013; Xu *et al.*, 2014a; Hu and Yu, 2014; Zhou *et al.*, 2015). Furthermore, *ABI5* expression is not just regulated by HY5 and pARR4 but also by FHY3 (Whitelam *et al.*, 1993; Oyama *et al.*, 1997; Yanovsky *et al.*, 2000; Osterlund *et al.*, 2000; Chen *et al.*, 2008; Wang *et al.*, 2011a; Tang *et al.*, 2013; Wang *et al.*, 2015). FHY3 was quantified in the phosphoproteome of *ahk1-3* and the wildtype but did not reveal differential phosphorylation after mannitol treatment. In darkness, FHY3 interacts with the Pr-form of phyA, is released upon light-induced conversion of phyA from the Pr- to the Pfr-form and is therefore part of the phyA signaling pathway (Saijo *et al.*, 2008). The phyA signaling pathway seems to play a minor role in AHK1-dependent signaling as components of the signaling pathway which lead to elongation growth were not even detected in the phosphoproteome of *ahk1-3* and the wildtype. Still it is possible, that in etiolated seedlings of *ahk1-3* lower levels of the transcription factor ATAF2 confer an enhanced expression of the genes *NIT2, BAS1* and *SOB7*, whereas NIT2 promotes auxin biosynthesis and BAS1 and SOB7 mediate the degradation of BL (Bartling *et al.*, 1992; Bartling *et al.*, 1994; Neff *et al.* 1999; Turk *et al.*, 2005; Delessert *et al.*, 2005; Huh *et al.* 2012; Peng *et al.*, 2015).

## 5.5 AHK1-dependent differential regulation of AHAs might cause the altered hypocotyl and root length in etiolated *ahk1* knock down seedlings.

Auxin and BR mediated alterations in cell elongation are known to be conveyed through alterations in the activity of $H^+$-ATPases (AHAs) (Takahashi *et al.*, 2012; Haruta *et al.*, 2015). According to the acid growth theory an increase of the activity of AHAs leads to an acidification of the apoplast, subsequently to cell wall loosening and therewith to the possibility of the cell to expand (Rayle and Cleland, 1992; Caesar *et al.*, 2011a; Wolf *et al.*, 2012). As shown in figure 4.36, AHA1 and AHA2 contribute to hypocotyl and root elongation during skotomorphogenic growth. Thereby, AHA1 which is expressed in root and shoot seems to play major roles in hypocotyl elongation as the root length in etiolated *aha1-6* seedlings is not altered in comparison to the wildtype Col-0 (Winter *et al.*, 2007). Due to shorter hypocotyls and roots in etiolated *aha2-4* seedlings, AHA2 might be involved in hypocotyl as well as in root elongation although AHA2 is mainly expressed in roots (Winter *et al.*, 2007).

In regard to the altered length of hypocotyls and roots of etiolated *ahk1* knock down seedlings an AHK1-dependent regulation of AHA activity can be assumed (fig. 4.34 and fig. 4.36). Thereby it has to be noted, that in Col-0 the phenotype is less consistent according to figure 4.15 and 4.36 but tends to

be opposing to Ws-2 and Nos-0. The opposite effect of the disruption of AHK1 in Ws-2 and Col-0 has already been discussed to be connected to Ws-2 being a natural phyD loss of function mutant (Aukerman *et al.,* 1997). As AHK1 as well as phyD might affect auxin- and BR- but also cytokinin-signaling the disruption of AHK1, phyD or both might lead to the opposing effect in skotomorphogenesis of *ahk1* knock down mutants.

Still, AHK1 might impact AHA activity. This hypothesis is supported by the finding that AHAs reveal at least the trend to be differentially phosphorylated in *ahk1-3* in comparison to the wildtype Ws-2 upon mock or mannitol treatment. The quantified phosphopeptides thereby identify the penultimate Thr-residue of AHA1 and AHA2 to show alterations in phosphorylation which is proposed to be the major switch of AHA activity (Nühse *et al.*, 2007; Palmgren *et al.*, 2001). However, the phosphoproteome of *ahk1-3* and Ws-2 revealed that the phosphorylation of these residues is not just affected by the presence or absence of AHK1 but also by mannitol-treatment. Thereby, AHA1 showed a slightly decreased phosphorylation at the penultimate Thr948 in light-grown seedlings of *ahk1-3* in comparison to the wildtype after treatment with mock and increased phosphorylation at this residue after treatment with mannitol indicating a general decrease of AHA1 activity in *ahk1-3* but an enhanced AHA1 activity during osmotic stress due to the absence of AHK1. This suggests a positive effect of AHK1 on AHA1 activity during normal growth but a negative effect of AHK1 on AHA1 activity during osmotic stress. AHA2 showed a slightly decreased phosphorylation at the penultimate Thr in *ahk1-3* after mock treatment like AHA1 but a higher decrease of phosphorylation after mannitol treatment indicating a generally decreased AHA2 activity in *ahk1-3* like described for AHA1 but in contrast to AHA1 a further decrease of AHA2 activity during osmotic stress. This in turn suggests an enhanced positive effect of AHK1 on AHA2 activity during osmotic stress. Previous studies of the phosphoproteome in Ler-0 and Col-0 upon treatment with the pathogen-associated molecular pattern (PAMP) flg22 have so far just shown cooperative regulation of AHA1- and AHA2-activity (Nühse *et al.*, 2000; Nühse *et al.*, 2007; Benshop *et al.*, 2007). The opposing differential phosphorylation of AHA1 and AHA2 at the penultimate Thr-residue in *ahk1-3* after mannitol treatment indicates a non-cooperative but specific and AHK1-dependent regulation of AHA1- and AHA2-activity during osmotic stress.

The shorter hypocotyls and roots of etiolated *ahk1* knock down mutants confirm the positive effect of AHK1 on AHA activity under control conditions as the absence of AHK1 leads to a decrease in hypocotyl and root length which indicates reduced AHA activity.

According to a higher contribution of AHA1 to hypocotyl than to root elongation, the negative effect of AHK1 on AHA1 activity during mannitol stress would suggest an increased activity of AHA1 in the absence of AHK1 and therefore an increased hypocotyl length of etiolated *ahk1* knock down seedlings at least in comparison to control conditions. In fact, this is the case as etiolated *ahk1* knock down seedlings reveal wildtype-like hypocotyl length after growth on mannitol-supplemented media (fig. 4.16). Furthermore, the contribution of AHA2 to hypocotyl and root elongation and the positive effect of AHK1 on AHA2 activity during mannitol stress would suggest a decreased activity of AHA2 in the absence of AHK1 during mannitol stress and therefore would imply a decreased hypocotyl and root length of etiolated *ahk1* knock down seedlings. In fact, etiolated *ahk1 kn*ock down seedlings reveal a

wildtype-like hypocotyl length (fig. 4.16). This might be explained by an additive effect of increased AHA1 activity and decreased AHA2 activity in the absence of AHK1 during mannitol stress.

In contrast to the expectation, etiolated *ahk1* knock down seedlings revealed no further decrease in root length during mannitol stress (fig. 4.16). This difference from the expectation might be explained by additional phosphorylation sites which are involved in the regulation of AHA activity. An additional phosphorylation site which is known to be involved in this regulation and which was quantified in the phosphoproteome of *ahk1-3* and Ws-2 but did not show differential phosphorylation neither after mock nor mannitol treatment is Ser899 in AHA1 as well as in AHA2 (gi:15234666) (Nühse *et al.*, 2007; Benshop *et al.*, 2007).

The inverse phosphorylation pattern of AHA1 and AHA2 at their penultimate Thr after mock and mannitol treatment, the phosphorylation at Ser899 which does not reveal any differential phosphorylation as well as the contribution of the other AHAs like AHA3 and AHA11 which also were quantified in the phosphoproteome of *ahk1-3* and Ws-2 might provide the explanation for the result of the AHA activity assay. In the AHA activity assay entire 14d old seedlings of *ahk1*-3 and the wildtype Ws-2 were harvested after mock or mannitol treatment for protein extraction and the determination of the change in inorganic phosphate without discrimination between roots and shoots. This assay did not reveal any change in the AHA activity between *ahk1-3* and the wildtype neither after mock nor after mannitol treatment (fig. 4.28). This raises the question whether AHA1 an AHA2 contribute to different processes in distinct signaling pathways. A first hint is given by the distinct phosphorylation of AHA1 and AHA2 at the penultimate Thr after flg22 or mannitol treatment (Nühse *et al.* 2007; Benshop *et al.* 2007). AHA1 is expressed in root and shoot whereas AHA2 is mainly expressed in roots like AHK1 (Winter *et al.*, 2007). AHA1 is known to contribute to stomatal opening in response to pathogen-attack and blue light whereas AHA2 is involved in the root architecture at least in response to different nitrogen supply (Liu *et al.*, 2009; Yamauchi *et al.*, 2016; Młodzińska *et al.*, 2015). This indicates a tissue and signal specific regulation of AHAs. This might be achieved by the binding of activating or inactivating factors which differ in response to a signal or in a tissue specific manner. Factors which are known to combine these characteristics are 14-3-3 proteins as they are known to bind to the phosphorylated penultimate Thr-residue of AHAs as a homo- or heterodimer and thereby enable the association of an active $H^+$-ATPase hexamer (Oecking and Jaspert 2009; Kanczewska *et al.*, 2005; Ottmann *et al.*, 2007; Aitken 1996). The expression of the different 14-3-3 proteins is not ubiquitous indicating that they are involved in specifying the signal transduction by activating or de-activating phosphorylated proteins specifically by enforcing a conformational change or by prohibiting or promoting the association with other proteins (Winter *et al.*, 2007; Oecking and Jaspert, 2009; Aitken 1996). For this specificity the composition of the 14-3-3 dimer might be important. Therefore it has to be noted, that the analysis of the phosphoproteome after metabolic labeling of *ahk1-3* and the wildtype Ws-2 after mock treatment revealed 14-3-3 upsilon (GRF5, AT5G16050) to show slightly decreased phosphorylation in *ahk1-3* in comparison to the wildtype at Ser2. This phosphorylation might have an influence on the dimerization of GRF5 with other 14-3-3 proteins as this phosphorylation is located closely to the residues 5-21 which are at least in mammalian 14-3-3 proteins known to be the site for dimerization with other 14-3-3 proteins (Aitken 1996; Xiao *et al.*, 1995; Liu *et al.*, 1995). In

consequence this might lead to a variety of differentially regulated phosphorylated proteins like AHAs which might then cause the respective phenotype.

Ser2 in GRF5 is predicted to be a putative phosphorylation site of casein kinases 2 (CK2) which are known to have diverse targets also comprising 14-3-3 proteins (Dinkel *et al.*, 2016; Oecking and Jaspert 2009; Mulekar and Huk 2014). CK2 are tetramers which are composed of two catalytic α-chains and two regulatory β-chains whereas in plants there are for genes respectively encoding α and β subunits (Litchfield, 2003; Salinas *et al.*, 2006). Recent studies suggest that CK2 is involved in the regulation of AHA activity (Lin *et al.*, 2015; Schiele, 2016). This might occur either through direct phosphorylation of AHAs although the known regulatory phosphorylation sites at the carboxy terminus of AHAs are not predicted to be putative phosphorylation sites of CK2 or by the phosphorylation of regulatory proteins like 14-3-3 proteins which influence their dimerization and activating properties (Jahn *et al.*, 1997; Fuglsang *et al.*, 1999; Oecking and Jaspert, 2009; Hayashi *et al.*, 2010; Haruta *et al.*, 2015; Dinkel *et al.*, 2016).

The analysis of the phosphoproteome after metabolic labeling of *ahk1-3* and the wildtype Ws-2 after treatment with mannitol revealed the decreased phosphorylation of the CK2 catalytic α-chain CKA3 (AT2G23080) in *ahk1-3* at Ser327 and Ser328 at the carboxy terminus. This AHK1-dependent differential phosphorylation might not alter the composition of the CK2 tetramer as the carboxy terminus of the α-subunit is not involved in tetramer formation (Litchfield, 2003). The carboxy terminus of CKA3 is predicted to contain a 14-3-3 binding motif comprising the CKA3 amino acid residues 329-333 with a low conservation score which nevertheless let assume that CK2 might regulate 14-3-3 dimerization whereas 14-3-3 dimers in turn might regulate the activity and specificity of CK2 (Dinkel *et al.*, 2016). This suggests that AHK1 has an impact on the phosphorylation and therefore on the activity and specificity of 14-3-3 proteins as well as on CK2 which influences the regulation of several developmental processes as well as adaptations to osmotic stress (Salinas *et al.* 2006; Mulekar and Huq, 2014).

The hypothesis of an AHK1-dependent regulation of CK2 activity might be further confirmed by the previously suggested change in HY5-levels in etiolated *ahk1* knock down lines as it has been assumed that HY5 phosphorylation which prevents HY5 from targeting to degradation by the ubiquitinating E3-ligase and which reduces HY5 DNA-binding affinity is mediated by a light-regulated CK2 (Hardtke *et al.*, 2000). Furthermore, the AHK1- and mannitol-dependent differential phosphorylation of Thr883 in AHK4 (gi:30677959) might also be mediated by a CK2 as well as it has been revealed as putative CK2 phosphorylation site (Dinkel *et al.* 2016). This indicates that an AHK1-dependent change in CK2 activity might cause the difference in cytokinin response of etiolated *ahk1* knock down seedlings.

## 5.6 PIPs do not show AHK1-dependent regulation

The putative AHK1-dependent contribution of 14-3-3 proteins to the regulation of developmental processes and adaption to osmotic stress might be further confirmed by the finding, that the analysis of the phosphoproteome of *ahk1-3* and the wildtype Ws-2 revealed alterations in the phosphorylation of several plasma membrane intrinsic proteins (PIPs) which belong to the gene family of aquaporins. The quantified phosphopeptides revealed alterations in the phosphorylation of Ser-residues at the carboxy terminus of the PIPs which are known to be involved in the regulation of PIP activity as the

phosphorylation of these residues favors the open pore conformation (Maurel *et al.*, 2015; Sjövall-Larsen *et al.*, 2006; Wu *et al.*, 2013). In mammals it has been shown, that 14-3-3 proteins bind to the phosphorylated carboxy terminus of PIPs in order to regulate their activity whereas it has to be noted that in plantal PIPs the binding of 14-3-3 dimers is predicted not at the PIPs carboxy terminus but at their respective loop B (Dinkel *et al.* 2016; Moeller *et al.*, 2016).

In addition to the regulation of PIP activity by 14-3-3 proteins it has been shown, that the phosphorylation of Ser280 and Ser283 in the carboxy terminus of PIP2A (AT3G53420) which is necessary for 14-3-3 binding might depend on the activity of $Ca^{2+}$-dependent kinases and SIRK1 (Sjövall-Larsen *et al.*, 2006; Wu *et al.*, 2013; Moeller *et al.*, 2016; Yaneff *et al.*, 2016; Wilson *et al.*, 2016). The analysis of the phosphoproteome of *ahk1-3* and Ws-2 revealed after mock as well as after mannitol treatment differential phosphorylation of several $Ca^{2+}$-dependent kinases as well as of SIRK1 (AT5G10020). This indicates that the AHK1-dependent differential phosphorylation of $Ca^{2+}$-dependent kinases and SIRK1 might lead to the altered phosphorylation of PIPs and therefore to a change in 14-3-3 binding and PIP activity (Wu *et al.* 2013; Maurel *et al.*, 2015; Wilson *et al.*, 2016).

However, even though the phosphoproteome of *ahk1-3* and the wildtype Ws-2 revealed differential phosphorylation of the PIPs at suggested regulatory phosphorylation sites, protoplasts of hypocotyls and roots of light-grown *ahk1* knock down seedlings did not show any difference in the water flux density how it was described for *sirk1* mutants when exposed to hypo-osmolar medium (Wu *et al.*, 2013). This indicates that there are no AHK1-dependent alterations in the water transport and therefore no change in the PIP activity. This discrepancy between the similar PIP activity and the altered phosphorylation might be explained by the inverse phosphorylation pattern of the PIPs how it was previously observed and described for AHA1 and AHA2. This means that the AHK1-dependent phosphorylation of PIPs which favors the open pore conformation and therefore leads to increased activity of PIPs might countervail the decreased activity of other PIPs which results in the determination of water flux densities which does not show any AHK1-dependent alteration (Maurel *et al.*, 2015).

## 5.7 AHK1 might not be a mechano-sensitive osmosensor

Conclusively, AHK1 impacts BR and cytokinin signaling as well as auxin, ABA and other hormone responses. In addition, AHK1 might work as positive regulator in osmotic stress. Although Kumar *et al.* (2013) suggested that AHK1 may not be the main osmosensor in plants this cannot be ruled out. Still, the signal perception might not occur in a mechano-sensitive manner how it was previously proposed (Urao *et al.*, 1999; Tran *et al.*, 2007; Wohlbach *et al.*, 2008). This is suggested by the homology model of the structure of the extracellular domain of AHK1 (fig. 4.4) which is most likely the site of signal perception but which is assumed to be stiff and therefore not susceptible for mechanical stimuli. Instead of the perception of a mechanical stimulus like a change in the turgor pressure a ligand is suggested to serve as signal for the induction of AHK1 activity which is due to the identification of a putative PAS domain in the extracellular domain of AHK1 (fig. 4.4). PAS domains are highly divergent at the primary sequence level but reveal a conserved three-dimensional architecture (Henry and Crosson, 2011). The prediction of this PAS-domain therefore suggests the binding of a small-molecule metabolite to AHK1 as this is a hallmark of the PAS-domain family (Henry and Crosson, 2011). Small-molecule metabolites which are already identified to bind to PAS-domains are for instance divalent

metal ions like $Mg^{2+}$ as well as $Ca^{2+}$, fatty acids and C4- and C6 carboxylate-containing substrates as well as heme, flavin or 4-hydroxycinnamic acid which are shown to be involved in sensing of light, oxygen and the redox-state of the cell (Henry and Crosson, 2011). It remains to be elucidated, which ligand binds to AHK1 and which signal is perceived.

Osmotic stress can be induced by several different factors like for instance altered water availability, flooding and therefore altered oxygen availability, dissolved ion content, abundance of other osmotically active substances, atmospheric humidity, temperature, wind speed as well as solar irradiance (Stephan *et al.*, 2016). This leads to the requirement of the ability of the plant to integrate several information of the nature of osmotic stress which necessitates the adjustment of different hormonal pathways (Suzuki *et al.*, 2016). In case AHK1 acts as an osmosensor, this might explain the diversity of pathways which are AHK1-dependently influenced upon mannitol treatment as well as the variety of different and sometimes inconsistent phenotypes of *ahk1* knock down lines when factors like temperature or the relative position to the light source varied.

According to this, it is not surprising that many proteins which are involved in calcium signaling reveal differential phosphorylation in *ahk1-3* in comparison to the wildtype after mock as well as after mannitol treatment. Calcium is known to act as second messenger in several developmental as well as in stress responsive processes which also includes the regulation of abscissic acid signaling (Dodd *et al.*, 2010; Guo *et al.*, 2002). Furthermore, previous studies revealed that osmotic stress induces a rise in intracellular $Ca^{2+}$ within seconds after stress application (Stephan *et al.*, 2016; Knight *et al.*, 1997; Shimomura *et al.*, 1962). This might occur through AHK1-dependent differential phosphorylation of $Ca^{+}$-transporters. In this regard it has been shown that rapid hyperosmotic-induced $Ca^{2+}$-responses involve plastidial KEA transporters indicating that KEA1, KEA2 and KEA3 contribute to the rapid intracellular increase in $Ca^{2+}$-levels upon osmotic stress (Stephan *et al.*, 2016). Thereby *kea1-2 kea2-2* and *kea3-1* showed altered hyperosmotic-induced $Ca^{2+}$-responses whereas this could not be shown for the quintuple mutant of the plasma-membrane localized putative mechano-sensitive channels of the MscS-like family *msl4,5,6,9,10* indicating a not mechanical induced increase of intracellular $Ca^{2+}$-levels (Stephan *et al.*, 2016; Wilson *et al.*, 2013). The phosphoproteome of *ahk1-3* and the wildtype Ws-2 showed that KEA2 (AT400630) actually comprises two phosphorylation sites but due to exclusive quantification of KEA2 in *ahk1-3* after mannitol treatment and in Ws-2 after mock treatment no conclusions could be drawn in regard to AHK1-dependent differential phosphorylation.

Nevertheless, an AHK1-dependent hyperosmotic-induced rise of intracellular $Ca^{2+}$-levels cannot be excluded. For that it would have been of tremendous interest to express the calcium sensor R-GECO1 in *ahk1* knock down mutants to investigate whether the $Ca^{2+}$-influx as well as its oscillation signature which is described to be changed upon osmotic stress is altered by the disruption of AHK1 (Keinath *et al.*, 2015; Zhao *et al.*, 2011; Tewson *et al.*, 2012; Monshausen *et al.*, 2009). Unfortunately the transformation of *ahk1-3* and *ahk1-4* with *A. thumefaciens* carrying a construct encoding R-GECO1 did not work and has to be repeated. Instead, crossings of Col-0 plants expressing the calcium sensors R-GECO1 and Yellow Cameleon 3.6 with *ahk1-5* and *ahk1-6* have been executed and F1-seeds have already been obtained (Behera *et al.*, 2013; Keinath *et al.* 2015; Zhao *et al.*, 2011). Still, these plant lines have to be progenated until first experiments can be performed but might then gain insight

whether the $Ca^{2+}$-signature upon osmotic stress is altered in *ahk1* knock down lines. If this is the case, this might indicate an AHK1-dependent regulation of $Ca^{2+}$-signaling which might in turn cause altered $Ca^{2+}$-dependent responses. This would include $Ca^{2+}$-dependent kinases, phosphatases and other proteins whose binding capabilities are altered by calcium (Dodd *et al.*, 2010). The phosphoproteome of *ahk1-3* and the wildtype suggests that this might be the case as for instance the $Na^+/H^+$-antiporter SOS1 (AT2G01980) revealed more phosphorylation in *ahk1-3* after mock treatment but wildtype-like phosphorylation after mannitol treatment. SOS1 has been shown to mediate salt stress adaption and to be regulated by the calcium sensing calcineurin B-like (CBL) protein SOS3 (AT5G24270) and the CBL-interacting protein kinase (CIPK) SOS2 (AT5G35410) (Dodd *et al.*, 2010; Gobert *et al.*, 2006; Liu *et al.*, 1997; Liu *et al.*, 2000). This indicates that SOS1 is differentially regulated in the absence of AHK1 which might lead to an altered oscillation of membrane potential and a change in cytosolic pH as SOS1 extrudes $Na^+$ but contemporaneously imports $H^+$ and thereby causes acidification of the cytosol (Dodd *et al.*, 2010; Gobert *et al.*, 2006; Liu *et al.*, 1997; Liu *et al.*, 2000). Cytosolic acidification in turn is for instance known to inactivate PIPs through protonation of a conserved His-residue in their cytoplasmic loop D which subsequently leads to a conformational change which stabilizes PIPs in their closed pore conformation (Törnroth-Horsefield *et al.*, 2006; Frick *et al.*, 2013; Maurel *et al.*, 2015). To overcome this effect, in *ahk1* knock down mutants the activity of CPKs and SIRK1 might be influenced as they have been shown to activate PIPs through phosphorylation (Sjövall-Larsen *et al.*, 2006; Wu *et al.*, 2013; Maurel *et al.*, 2015). This might lead to a change in the maintenance of turgor pressure in *ahk1* knock down lines. An involvement of AHK1 in this process can be assumed due to recent findings which reveal weaknesses of *ahk1* knock down lines to adjust turgor pressure after drought stress (Gerhard Obermeyer, unpublished, data not shown). This reveals the importance of AHK1 in integrating diverse signals to a proper adaption of the plant to different stresses.

## 5.8 AHK1 contributes to plant immunity through interaction with BAK1

Still, one main pathway of AHK1 signaling might comprise the interaction of AHK1 with BAK1 which might influence the BAK1-dependent transphosphorylation pattern of other receptor kinases (Wang *et al.*, 2008; Wang *et al.*, 2014a). That the phosphorylation state of BAK1-interacting kinases is changed in an AHK1-dependent manner has already been revealed by the analysis of the phosphoproteome of *ahk1-3* and the wildtype Ws-2 after mock and after mannitol treatment. In addition to the differential phosphorylation of BRI1, also the BAK1-interacting receptor like kinase BIR1 (fig. 5.1) and several other kinases of the LRR protein kinase family revealed AHK1-dependent differential phosphorylation including the PAMP-induced receptor FLS2 (fig. 5.1; appendix A33, A34, A35). The genotype Ws-0 has been shown to carry a non-functional *FLS2* allele, whereas this has so far not been revealed in Ws-2 (Vetter *et al.*, 2012; Zipfel *et al.*, 2004). A physiological connection between AHK1, BAK1 and the response to pathogen attack could be revealed in a pathogen assay with *Alternaria brassicicola* (fig. 4.37). Like in several other phenotyping experiments opposing phenotypes of *ahk1* knock down mutants could be observed due to different replicates. Nevertheless, the double mutants *bri1-5 ahk1-3* and *bak1-1 ahk1-3* revealed a tendency to react differently to pathogen attack than the respective single mutants. This effect has to be investigated more detailed under better defined growth conditions as it was observed that the result of the pathogen assay is highly influenced by atmospheric humidity.

This is due to a more sensitive reaction of the Ws-2 ecotype in comparison to Col-0 to atmospheric humidity which has not been described in previous studies but observed without any quantitation or precise documentation in the experiments of this study. For example it has been observed that growth on plates which in general comprises a higher atmospheric humidity led to the induction of flowering of Ws-2 two to three weeks after germination. In addition it was observed that high atmospheric humidity leads to curling or the turn of the upside down of the leaves of Ws-2 (data not shown). High atmospheric humidity in the pathogen assay is needed to open the stomata before the plants can be infected with spores of *Alternaria brassicicola*. For that reason the atmospheric humidity is upregulated by watering and the covering of the tray in which the plants are grown with a hood. Therefore the opposing phenotypes of *ahk1* knock down mutants in the different replicates of the pathogen assays might depend on not defined atmospheric humidity. As recent findings of Gerhard Obermeyer indicate weaknesses of *ahk1* to re-adjust turgor pressure after osmotic stress (data not shown), the exact atmospheric humidity and the thereby applied osmotic stress might highly influence the experimental result. According to that, this indicates that the BAK1-dependent reaction of the plant to pathogen attack is influenced by AHK1 and that the integration of the response to pathogen attack and to osmotic stress occurs in a BAK1-dependent manner.

The direct interaction of BAK1 with AHAs and the ion channel CNGC17, its influence on several signaling pathways and the possibility that BAK1 integrates these signals through mediating signal specific transphosphorylation patterns in association with its interaction partners suggests a supercomplex of BAK1, AHAs and ion channels like CNGC17 as core complex which adjusts ion homeostasis and membrane potential and which is accessed and differentially regulated by various receptor kinases (Dodd *et al.*, 2010; Caesar *et al.*, 2011a; Wolf *et al.*, 2012; Wang *et al.*, 2014a; Ladwig *et al.*, 2015).

The finding of differential phosphorylation in *ahk1-3* in comparison to the wildtype of LRR protein kinase family proteins, CNGC7 (AT1G155900) and other plasma membrane localized ion channels, AHAs as well as differential phosphorylation even of wall-associated kinases (WAK4=AT1G21210; WAK5=AT1G21230), which are transmembrane proteins with a cytoplasmic Ser/Thr kinase domain, which can be covalently linked to pectin in the cell wall, which are assumed to be receptors for promoting the cell-wall integrity pathway and which are known to be connected to BAK1 signaling through the revealed interaction of BAK1 with RLP44 (AT3G49750) which in turn interacts with WAK1 (AT1G21250), as well as the respective phenotypes further confirm the existence of such a supercomplex (Verica and He, 2002; Wolf *et al.*, 2012). The signal and tissue specific activity and composition of this supercomplex might be very interesting in regard to the integration of different signals and the specificity of plant response. The contribution of AHK1 in the signaling pathway of this supercomplex especially in regard to osmotic stress regulation might be furthermore interesting for the basic understanding of the plant's adaptation processes and later on for the development of plants which are more resistant to osmotic stress.

## 5.9 AHK1 might be an osmosensor and therefore impacts many hormonal signaling pathways

Conclusively, it remains to be elucidated which signal is perceived by AHK1. Still, the signal might be osmotic stress itself or a signal which is released upon osmotic stress and which has not yet been identified. Osmotic stress can occur in different forms which require fine-tuning and adaptation of all developmental processes. The phosphoproteome of *ahk1-3* and the wildtype Ws-2 in combination with further phenotyping studies revealed the cross-talk of AHK1-dependent signaling with several hormonal pathways like the brassinosteroid, cytokinin, auxin and abscissic acid signaling pathway. Thus, AHK1 might also influence the other hormonal pathways. This might work through the already known cross-talk of signaling pathways as well as through AHK1-dependent alterations in phophorylation patterns (Nemhauser *et al.*, 2006). For instance the PP2C family protein AP2C1 (AT2G30020) which revealed increased phosphorylation in *ahk1-3* after mannitol treatment is known to modulate innate immunity, jasmonic acid and ethylene levels (Schweighofer *et al.*, 2007). Ethylene signaling might be directly regulated by AHK1-dependent phosphorylation of the ethylene receptor EIN4 (AT3G04580) (table 4.1; Hua *et al.*, 1998). Furthermore, the receptor-like protein kinase FERONIA (FER, AT3G51550) showed decreased phosphorylation in *ahk1-3* in comparison to the wildtype after mock treatment and no differential phosphorylation after mannitol treatment (appendix A35). FER is known to act as receptor kinase for the peptide hormone called rapid alkalinization factor (RALF) and to control the production of high levels of reactive oxygen species and RALF-induced calcium spikes which are involved in root growth inhibition (Chen *et al.*, 2016a; Duan *et al.*, 2014; Haruta *et al.*, 2014; Yu *et al.*, 2014). In addition it is known that FER phosphorylation is inhibited by interaction with the PP2C phosphatase ABI2 which mediates cross-talk between RALF and abscissic acid and changes the expression levels of *RD29B*, *RAB18* as well as *ABI5* (Chen *et al.*, 2016a). Furthermore, increased levels of gibberellic acid in darkness lead to the release of BZR1 and PIF proteins from DELLA proteins which enables BZR1 and PIFs to form active heterodimers to regulate genes which are involved in hypocotyl growth (Bae and Choi, 2008; Feng *et al.*, 2008; de Lucas *et al.*, 2008; Bai *et al.*, 2012b; Jaillais and Vert, 2012). This indicates that gibberellic acid might be involved in AHK1-dependent BZR1 regulation as well. Several proteins of these pathways were quantified and found to be differentially phosphorylated in *ahk1-3* in comparison to the wildtype Ws-2. How the AHK1-dependent signaling pathway upon osmotic stress takes place, how it influences all these hormonal signaling pathways and whether it occurs through interaction with BAK1, AHPs or both remains to be elucidated and verified.

# 6 REFERENCES


Aitken A, Howell S, Jones D, Madrazo J, Martin H, Patel Y, Robinson K. Post-translationally modified 14-3-3 isoforms and inhibition of protein kinase C. Mol Cell Biochem. 1995 Aug-Sep;149-150:41-9.

Aitken A. 14-3-3 and its possible role in co-ordinating multiple signaling pathways. Trends Cell Biol. 1996 Sep;6(9):341-7.

Al-Sady B, Ni W, Kircher S, Schäfer E, Quail PH. Photoactivated phytochrome induces rapid PIF3 phosphorylation prior to proteasome-mediated degradation. Mol Cell. 2006 Aug 4;23(3):439-46.

Anantharaman V, Aravind L. The CHASE domain: a predicted ligand-binding module in plant cytokinin receptors and other eukaryotic and bacterial receptors. Trends Biochem Sci. 2001 Oct;26(10):579-82.

Appleby JL, Parkinson JS, Bourret RB. Signal transduction via the multi-step phosphorelay: not necessarily a road less traveled. Cell. 1996 Sep 20;86(6):845-8. Review.

Argyros RD, Mathews DE, Chiang YH, Palmer CM, Thibault DM, Etheridge N, Argyros DA, Mason MG, Kieber JJ, Schaller GE. Type B response regulators of Arabidopsis play key roles in cytokinin signaling and plant development. Plant Cell. 2008 Aug;20(8):2102-16.

Arsova B, Kierszniowska S, Schulze WX. The use of heavy nitrogen in quantitative proteomics experiments in plants. Trends Plant Sci. 2012 Feb;17(2):102-12.

Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J. MAP kinase signalling cascade in Arabidopsis innate immunity. Nature. 2002 Feb 28;415(6875):977-83.

Aukerman MJ, Hirschfeld M, Wester L, Weaver M, Clack T, Amasino RM, Sharrock RA. A deletion in the PHYD gene of the Arabidopsis Wassilewskija ecotype defines a role for phytochrome D in red/far-red light sensing. Plant Cell. 1997 Aug;9(8):1317-26.

Bae G, Choi G. Decoding of light signals by plant phytochromes and their interacting proteins. Annu Rev Plant Biol. 2008;59:281-311. Review.

Bai MY, Fan M, Oh E, Wang ZY. A triple helix-loop-helix/basic helix-loop-helix cascade controls cell elongation downstream of multiple hormonal and environmental signaling pathways in Arabidopsis. Plant Cell. 2012a Dec;24(12):4917-29.

Bai MY, Shang JX, Oh E, Fan M, Bai Y, Zentella R, Sun TP, Wang ZY. Brassinosteroid, gibberellin and phytochrome impinge on a common transcription module in Arabidopsis. Nat Cell Biol. 2012b Aug;14(8):810-7.

Balcerowicz M, Fittinghoff K, Wirthmueller L, Maier A, Fackendahl P, Fiene G, Koncz C, Hoecker U. Light exposure of Arabidopsis seedlings causes rapid de-stabilization as well as selective post-translational inactivation of the repressor of photomorphogenesis SPA2. Plant J. 2011 Mar;65(5):712-23.

Bartling D, Seedorf M, Mithöfer A, Weiler EW. Cloning and expression of an Arabidopsis nitrilase which can convert indole-3-acetonitrile to the plant hormone, indole-3-acetic acid. Eur J Biochem. 1992 Apr 1;205(1):417-24.

Bartling D, Seedorf M, Schmidt RC, Weiler EW. Molecular characterization of two cloned nitrilases from Arabidopsis thaliana: key enzymes in biosynthesis of the plant hormone indole-3-acetic acid. Proc Natl Acad Sci U S A. 1994 Jun 21;91(13):6021-5.

Bashline L, Lei L, Li S, Gu Y. Cell wall, cytoskeleton, and cell expansion in higher plants. Mol Plant. 2014 Apr;7(4):586-600. Review.

Bauer J, Reiss K, Veerabagu M, Heunemann M, Harter K, Stehle T. Structure-function analysis of Arabidopsis thaliana histidine kinase AHK5 bound to its cognate phosphotransfer protein AHP1. Mol Plant. 2013 May;6(3):959-70.

Behera S, Krebs M, Loro G, Schumacher K, Costa A, Kudla J. Ca2+ imaging in plants using genetically encoded Yellow Cameleon Ca2+ indicators. Cold Spring Harb Protoc. 2013 Aug 1;2013(8):700-3.

Benjamini, Y., Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Royal Stat. Soc. 57: 289-300.

Benschop JJ, Mohammed S, O'Flaherty M, Heck AJ, Slijper M, Menke FL. Quantitative phosphoproteomics of early elicitor signaling in Arabidopsis. Mol Cell Proteomics. 2007 Jul;6(7):1198-214.

Bleecker AB, Estelle MA, Somerville C, Kende H. Insensitivity to Ethylene Conferred by a Dominant Mutation in Arabidopsis thaliana. Science. 1988 Aug 26;241(4869):1086-9.

Bleecker AB, Kende H. Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol. 2000;16:1-18. Review.

Bolle C, Koncz C, Chua NH. PAT1, a new member of the GRAS family, is involved in phytochrome A signal transduction. Genes Dev. 2000 May 15;14(10):1269-78.

Borden KL, Freemont PS. The RING finger domain: a recent example of a sequence-structure family. Curr Opin Struct Biol. 1996 Jun;6(3):395-401. Review.

Bosu DR, Kipreos ET. Cullin-RING ubiquitin ligases: global regulation and activation cycles. Cell Div. 2008 Feb 18;3:7.

Boudsocq M, Droillard MJ, Barbier-Brygoo H, Laurière C. Different phosphorylation mechanisms are involved in the activation of sucrose non-fermenting 1 related protein kinases 2 by osmotic stresses and abscisic acid. Plant Mol Biol. 2007 Mar;63(4):491-503.

Briggs WR, Christie JM. Phototropins 1 and 2: versatile plant blue-light receptors. Trends Plant Sci. 2002 May;7(5):204-10. Review.

Burg MB, Ferraris JD, Dmitrieva NI. Cellular response to hyperosmotic stresses. Physiol Rev. 2007 Oct;87(4):1441-74. Review.

Caesar K. Fluoreszenzmikroskopische Analyse pflanzlicher Transmembranrezeptoren. Dissertation. 2010

Caesar K, Elgass K, Chen Z, Huppenberger P, Witthöft J, Schleifenbaum F, Blatt MR, Oecking C, Harter K. A fast brassinolide-regulated response pathway in the plasma membrane of Arabidopsis thaliana. Plant J. 2011a May;66(3):528-40.

Caesar K, Thamm AM, Witthöft J, Elgass K, Huppenberger P, Grefen C, Horak J, Harter K. Evidence for the localization of the Arabidopsis cytokinin receptors AHK3 and AHK4 in the endoplasmic reticulum. J Exp Bot. 2011b Nov;62(15):5571-80.

Cai Z, Liu J, Wang H, Yang C, Chen Y, Li Y, Pan S, Dong R, Tang G, Barajas-Lopez Jde D, Fujii H, Wang X. GSK3-like kinases positively modulate abscisic acid signaling through phosphorylating subgroup III SnRK2s in Arabidopsis. Proc Natl Acad Sci U S A. 2014 Jul 1;111(26):9651-6.

Cao Y, Tanaka K, Nguyen CT, Stacey G. Extracellular ATP is a central signaling molecule in plant stress responses. Curr Opin Plant Biol. 2014 Aug;20:82-7. Review.

Carles C, Bies-Etheve N, Aspart L, Léon-Kloosterziel KM, Koornneef M, Echeverria M, Delseny M. Regulation of Arabidopsis thaliana Em genes: role of ABI5. Plant J. 2002 May;30(3):373-83.

Casal JJ, Sanchez RA. Phytochromes and seed germination. Seed Sci. Res. 1998 8:317-29

Cashmore AR. Cryptochromes: enabling plants and animals to determine circadian time. Cell. 2003 Sep 5;114(5):537-43. Review.

Casino P, Rubio V, Marina A. Structural insight into partner specificity and phosphoryl transfer in two-component signal transduction. Cell. 2009 Oct 16;139(2):325-36.

Causier B, Ashworth M, Guo W, Davies B. The TOPLESS interactome: a framework for gene repression in Arabidopsis. Plant Physiol. 2012 Jan;158(1):423-38.

Chang C, Kwok SF, Bleecker AB, Meyerowitz EM. Arabidopsis ethylene-response gene ETR1: similarity of product to two-component regulators. Science. 1993 Oct 22;262(5133):539-44.

Chattopadhyay S, Ang LH, Puente P, Deng XW, Wei N. Arabidopsis bZIP protein HY5 directly interacts with light-responsive promoters in mediating light control of gene expression. Plant Cell. 1998 May;10(5):673-83.

Chaves I, Pokorny R, Byrdin M, Hoang N, Ritz T, Brettel K, Essen LO, van der Horst GT, Batschauer A, Ahmad M. The cryptochromes: blue light photoreceptors in plants and animals. Annu Rev Plant Biol. 2011;62:335-64.

Chen H, Huang X, Gusmaroli G, Terzaghi W, Lau OS, Yanagawa Y, Zhang Y, Li J,Lee JH, Zhu D, Deng XW. Arabidopsis CULLIN4-damaged DNA binding protein 1 interacts with CONSTITUTIVELY PHOTOMORPHOGENIC1-SUPPRESSOR OF PHYA complexes to regulate photomorphogenesis and flowering time. Plant Cell. 2010 Jan;22(1):108-23.

Chen J, Yu F, Liu Y, Du C, Li X, Zhu S, Wang X, Lan W, Rodriguez PL, Liu X, Li D, Chen L, Luan S. FERONIA interacts with ABI2-type phosphatases to facilitate signaling cross-talk between abscisic acid and RALF peptide in Arabidopsis. Proc Natl Acad Sci U S A. 2016a Sep 13;113(37):E5519-27.

Chen M, Chory J, Fankhauser C. Light signal transduction in higher plants. Annu Rev Genet. 2004;38:87-117. Review.

Chen NZ, Zhang XQ, Wei PC, Chen QJ, Ren F, Chen J, Wang XC. AtHAP3b plays a crucial role in the regulation of flowering time in Arabidopsis during osmotic stress. J Biochem Mol Biol. 2007 Nov 30;40(6):1083-9.

Chen S, Lory N, Stauber J, Hoecker U. Photoreceptor Specificity in the Light-Induced and COP1-Mediated Rapid Degradation of the Repressor of Photomorphogenesis SPA2 in Arabidopsis. PLoS Genet. 2015 Sep 14;11(9):e1005516.

Chen S, Wirthmueller L, Stauber J, Lory N, Holtkotte X, Leson L, Schenkel C, Ahmad M, Hoecker U. The functional divergence between SPA1 and SPA2 in Arabidopsis photomorphogenesis maps primarily to the respective N-terminal kinase-like domain. BMC Plant Biol. 2016b Jul 22;16(1):165.

Cheng Y, Dai X, Zhao Y. Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in Arabidopsis. Genes Dev. 2006 Jul 1;20(13):1790-9.

Chini A, Fonseca S, Fernández G, Adie B, Chico JM, Lorenzo O, García-Casado G, López-Vidriero I, Lozano FM, Ponce MR, Micol JL, Solano R. The JAZ family of repressors is the missing link in jasmonate signalling. Nature. 2007 Aug 9;448(7154):666-71.

Choi H, Oh E. PIF4 Integrates Multiple Environmental and Hormonal Signals for Plant Growth Regulation in Arabidopsis. Mol Cells. 2016 Aug 31;39(8):587-93.

Choi J, Tanaka K, Cao Y, Qi Y, Qiu J, Liang Y, Lee SY, Stacey G. Identification of a plant receptor for extracellular ATP. Science. 2014 Jan 17;343(6168):290-4.

Chory J, Reinecke D, Sim S, Washburn T, Brenner M. A Role for Cytokinins in De-Etiolation in Arabidopsis (det Mutants Have an Altered Response to Cytokinins). Plant Physiol. 1994 Feb;104(2):339-347.

Christie JM, Blackwood L, Petersen J, Sullivan S. Plant flavoprotein photoreceptors. Plant Cell Physiol. 2015 Mar;56(3):401-13. Review.

Clough RC, Vierstra RD. Phytochrome degradation. Plant Cell Environ. 1997; 20:713-721

Clough SJ, Bent AF. Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. 1998 Dec;16(6):735-43.

Colón-Carmona A, Chen DL, Yeh KC, Abel S. Aux/IAA proteins are phosphorylated by phytochrome in vitro. Plant Physiol. 2000 Dec;124(4):1728-38.

Cominelli E, Galbiati M, Tonelli C. Integration of water stress response: Cell expansion and cuticle deposition in Arabidopsis thaliana. Plant Signal Behav. 2008 Aug;3(8):556-7.

Cox J, Mann M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nat Biotechnol. 2008 Dec;26(12):1367-72.

Cox J, Neuhauser N, Michalski A, Scheltema RA, Olsen JV, Mann M. Andromeda: a peptide search engine integrated into the MaxQuant environment. J Proteome Res. 2011 Apr 1;10(4):1794-805.

Crowell EF, Bischoff V, Desprez T, Rolland A, Stierhof YD, Schumacher K, Gonneau M, Höfte H, Vernhettes S. Pausing of Golgi bodies on microtubules regulates secretion of cellulose synthase complexes in Arabidopsis. Plant Cell. 2009 Apr;21(4):1141-54.

Curtis MD, Grossniklaus U. A gateway cloning vector set for high-throughput functional analysis of genes in planta. Plant Physiol. 2003 Oct;133(2):462-9.

D'Agostino IB, Deruère J, Kieber JJ. Characterization of the response of the Arabidopsis response regulator gene family to cytokinin. Plant Physiol. 2000 Dec;124(4):1706-17.

Dai M, Xue Q, Mccray T, Margavage K, Chen F, Lee JH, Nezames CD, Guo L, Terzaghi W, Wan J, Deng XW, Wang H. The PP6 phosphatase regulates ABI5 phosphorylation and abscisic acid signaling in Arabidopsis. Plant Cell. 2013 Feb;25(2):517-34.

Dai M, Zhang C, Kania U, Chen F, Xue Q, McCray T, Li G, Qin G, Wakeley M, Terzaghi W, Wan J, Zhao Y, Xu J, Friml J, Deng XW, Wang H. A PP6-type phosphatase holoenzyme directly regulates PIN phosphorylation and auxin efflux in Arabidopsis. Plant Cell. 2012 Jun;24(6):2497-514.

Darwish E, Testerink C, Khalil M, El-Shihy O, Munnik T. Phospholipid signaling responses in salt-stressed rice leaves. Plant Cell Physiol. 2009 May;50(5):986-97.

de Lucas M, Davière JM, Rodríguez-Falcón M, Pontin M, Iglesias-Pedraz JM, Lorrain S, Fankhauser C, Blázquez MA, Titarenko E, Prat S. A molecular framework for light and gibberellin control of cell elongation. Nature. 2008 Jan 24;451(7177):480-4.

De Smet S, Cuypers A, Vangronsveld J, Remans T. Gene Networks Involved inHormonal Control of Root Development in Arabidopsis thaliana: A Framework for Studying Its Disturbance by Metal Stress. Int J Mol Sci. 2015 Aug14;16(8):19195-224. Review.

Delessert C, Kazan K, Wilson IW, Van Der Straeten D, Manners J, Dennis ES, Dolferus R. The transcription factor ATAF2 represses the expression of pathogenesis-related genes in Arabidopsis. Plant J. 2005 Sep;43(5):745-57.

Delker C, Sonntag L, James GV, Janitza P, Ibañez C, Ziermann H, Peterson T, Denk K, Mull S, Ziegler J, Davis SJ, Schneeberger K, Quint M. The DET1-COP1-HY5 pathway constitutes a multipurpose signaling module regulating plant photomorphogenesis and thermomorphogenesis. Cell Rep. 2014 Dec 24;9(6):1983-9.

Deribe YL, Pawson T, Dikic I. Post-translational modifications in signal integration. Nat Struct Mol Biol. 2010 Jun;17(6):666-72.

Desikan R, Horák J, Chaban C, Mira-Rodado V, Witthöft J, Elgass K, Grefen C, Cheung MK, Meixner AJ, Hooley R, Neill SJ, Hancock JT, Harter K. The histidine kinase AHK5 integrates endogenous and environmental signals in Arabidopsis guard cells. PLoS One. 2008 Jun 18;3(6):e2491.

Dharmasiri N, Dharmasiri S, Estelle M. The F-box protein TIR1 is an auxin receptor. Nature. 2005 May 26;435(7041):441-5.

Di DW, Zhang C, Guo GQ. Involvement of secondary messengers and small organic molecules in auxin perception and signaling. Plant Cell Rep. 2015 Jun;34(6):895-904.

Dihazi H, Kessler R, Eschrich K. High osmolarity glycerol (HOG) pathway-induced phosphorylation and activation of 6-phosphofructo-2-kinase are essential for glycerol accumulation and yeast cell proliferation under hyperosmotic stress. J Biol Chem. 2004 Jun 4;279(23):23961-8. Epub 2004 Mar 22.

Dijkwel PP, Huijser C, Weisbeek PJ, Chua NH, Smeekens SC. Sucrose control of phytochrome A signaling in Arabidopsis. Plant Cell. 1997 Apr;9(4):583-95.

Dinesh DC, Kovermann M, Gopalswamy M, Hellmuth A, Calderón Villalobos LI, Lilie H, Balbach J, Abel S. Solution structure of the PsIAA4 oligomerization domain reveals interaction modes for transcription factors in early auxin response. Proc Natl Acad Sci U S A. 2015 May 12;112(19):6230-5.

Dinkel H, Van Roey K, Michael S, Kumar M, Uyar B, Altenberg B, Milchevskaya V, Schneider M, Kühn H, Behrendt A, Dahl SL, Damerell V, Diebel S, Kalman S, Klein S, Knudsen AC, Mäder C, Merrill S, Staudt A, Thiel V, Welti L, Davey NE, Diella F, Gibson TJ. ELM 2016--data update and new functionality of the eukaryotic linear motif resource. Nucleic Acids Res. 2016 Jan 4;44(D1):D294-300.

Domagalska MA, Sarnowska E, Nagy F, Davis SJ. Genetic analyses of interactions among gibberellin, abscisic acid, and brassinosteroids in the control of flowering time in Arabidopsis thaliana. PLoS One. 2010 Nov 17;5(11):e14012.

Domagalska MA, Schomburg FM, Amasino RM, Vierstra RD, Nagy F, Davis SJ. Attenuation of brassinosteroid signaling enhances FLC expression and delays flowering. Development. 2007 Aug;134(15):2841-50.

Donaldson L, Meier S, Gehring C. The arabidopsis cyclic nucleotide interactome. Cell Commun Signal. 2016 May 11;14(1):10.

Dortay H, Gruhn N, Pfeifer A, Schwerdtner M, Schmülling T, Heyl A. Toward an interaction map of the two-component signaling pathway of Arabidopsis thaliana. J Proteome Res. 2008 Sep;7(9):3649-60.

Dortay H, Mehnert N, Bürkle L, Schmülling T, Heyl A. Analysis of protein interactions within the cytokinin-signaling pathway of Arabidopsis thaliana. FEBS J. 2006 Oct;273(20):4631-44.

Drechsler T. Expression and purification of specific protein fragments of AHK1, AHK5 and AHP1 for antibody production and biochemical characterization. Masterarbeit. 2015

Droillard MJ, Boudsocq M, Barbier-Brygoo H, Laurière C. Involvement of MPK4 in osmotic stress response pathways in cell suspensions and plantlets of Arabidopsis thaliana: activation by hypoosmolarity and negative role in hyperosmolarity tolerance. FEBS Lett. 2004 Sep 10;574(1-3):42-8.

Du W, Lin H, Chen S, Wu Y, Zhang J, Fuglsang AT, Palmgren MG, Wu W, Guo Y. Phosphorylation of SOS3-like calcium-binding proteins by their interacting SOS2-like protein kinases is a common regulatory mechanism in Arabidopsis. Plant Physiol. 2011 Aug;156(4):2235-43

Duan L, Dietrich D, Ng CH, Chan PM, Bhalerao R, Bennett MJ, Dinneny JR. Endodermal ABA signaling promotes lateral root quiescence during salt stress in Arabidopsis seedlings. Plant Cell. 2013 Jan;25(1):324-41.

Duan Q, Kita D, Johnson EA, Aggarwal M, Gates L, Wu HM, Cheung AY. Reactive oxygen species mediate pollen tube rupture to release sperm for fertilization in Arabidopsis. Nat Commun. 2014;5:3129.

Evered C, Majevadia B, Thompson DS. Cell wall water content has a direct effect on extensibility in growing hypocotyls of sunflower (Helianthus annuus L.). J Exp Bot. 2007;58(12):3361-71.

Fan XY, Sun Y, Cao DM, Bai MY, Luo XM, Yang HJ, Wei CQ, Zhu SW, Sun Y, Chong K, Wang ZY. BZS1, a B-box protein, promotes photomorphogenesis downstream of both brassinosteroid and light signaling pathways. Mol Plant. 2012 May;5(3):591-600.

Fankhauser C, Christie JM. Plant phototropic growth. Curr Biol. 2015 May 4;25(9):R384-9. Review.

Fankhauser C. Phytochromes as light-modulated protein kinases. Semin Cell Dev Biol. 2000 Dec;11(6):467-73. Review.

Farkas I, Dombrádi V, Miskei M, Szabados L, Koncz C. Arabidopsis PPP family of serine/threonine phosphatases. Trends Plant Sci. 2007 Apr;12(4):169-76. Epub 2007 Mar 23. Review.

Favory JJ, Stec A, Gruber H, Rizzini L, Oravecz A, Funk M, Albert A, Cloix C, Jenkins GI, Oakeley EJ, Seidlitz HK, Nagy F, Ulm R. Interaction of COP1 and UVR8 regulates UV-B-induced photomorphogenesis and stress acclimation in Arabidopsis. EMBO J. 2009 Mar 4;28(5):591-601.

Felle H, Brummer B, Bertl A, Parish RW. Indole-3-acetic acid and fusicoccin cause cytosolic acidification of corn coleoptile cells. Proc Natl Acad Sci U S A. 1986 Dec;83(23):8992-5.

Felle HH. pH: Signal and Messenger in Plant Cells. Plant biol. 2001 Oct 02;3:577-591

Feng S, Martinez C, Gusmaroli G, Wang Y, Zhou J, Wang F, Chen L, Yu L, Iglesias-Pedraz JM, Kircher S, Schäfer E, Fu X, Fan LM, Deng XW. Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. Nature. 2008 Jan 24;451(7177):475-9.

Fernando VC, Schroeder DF. Arabidopsis DDB1-CUL4 E3 ligase complexes in det1 salt/osmotic stress resistant germination. Plant Signal Behav. 2016 Aug 22:0.

Fields S, Song O. A novel genetic system to detect protein-protein interactions. Nature. 1989 Jul 20;340(6230):245-6.

Finkelstein R. Abscisic Acid synthesis and response. Arabidopsis Book. 2013 Nov 1;11:e0166.

Finkelstein RR, Mutations at two new Arabidopsis ABA response loci are similar to the abi3 mutations. Plant J. 1994 5:765-771

Finkelstein RR, Wang ML, Lynch TJ, Rao S, Goodman HM. The Arabidopsis abscisic acid response locus ABI4 encodes an APETALA 2 domain protein. Plant Cell. 1998 Jun;10(6):1043-54.

Fittinghoff K, Laubinger S, Nixdorf M, Fackendahl P, Baumgardt RL, Batschauer A, Hoecker U. Functional and expression analysis of Arabidopsis SPA genes during seedling photomorphogenesis and adult growth. Plant J. 2006 Aug;47(4):577-90.

Franklin KA, Lee SH, Patel D, Kumar SV, Spartz AK, Gu C, Ye S, Yu P, Breen G, Cohen JD, Wigge PA, Gray WM. Phytochrome-interacting factor 4 (PIF4) regulates auxin biosynthesis at high temperature. Proc Natl Acad Sci U S A. 2011 Dec 13;108(50):20231-5.

Frick A, Järvå M, Törnroth-Horsefield S. Structural basis for pH gating of plant aquaporins. FEBS Lett. 2013 Apr 2;587(7):989-93.

Fu ZQ, Yan S, Saleh A, Wang W, Ruble J, Oka N, Mohan R, Spoel SH, Tada Y, Zheng N, Dong X. NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. Nature. 2012 May 16;486(7402):228-32.

Fuglsang AT, Visconti S, Drumm K, Jahn T, Stensballe A, Mattei B, Jensen ON, Aducci P, Palmgren MG. Binding of 14-3-3 protein to the plasma membrane H(+)-ATPase AHA2 involves the three C-terminal residues Tyr(946)-Thr-Val and requires phosphorylation of Thr(947). J Biol Chem. 1999 Dec 17;274(51):36774-80.

Fujino K, Koda Y, Kikuta Y. Reorientation of cortical microtubules in the sub-apical region during tuberization in single-node stem segments of potato in culture. Plant CellPhysiol. 1995 36(5): 891-895

Fujita Y, Nakashima K, Yoshida T, Katagiri T, Kidokoro S, Kanamori N, Umezawa T, Fujita M, Maruyama K, Ishiyama K, Kobayashi M, Nakasone S, Yamada K, Ito T, Shinozaki K, Yamaguchi-Shinozaki K. Three SnRK2 protein kinases are the main positive regulators of abscisic acid signaling in response to water stress in Arabidopsis. Plant Cell Physiol. 2009 Dec;50(12):2123-32.

Furuya T, Matsuoka D, Nanmori T. Phosphorylation of Arabidopsis thaliana MEKK1 via Ca(2+) signaling as a part of the cold stress response. J Plant Res. 2013 Nov;126(6):833-40.

Gagne JM, Downes BP, Shiu SH, Durski AM, Vierstra RD. The F-box subunit of the SCF E3 complex is encoded by a diverse superfamily of genes in Arabidopsis. Proc Natl Acad Sci U S A. 2002 Aug 20;99(17):11519-24.

Gampala SS, Kim TW, He JX, Tang W, Deng Z, Bai MY, Guan S, Lalonde S, Sun Y, Gendron JM, Chen H, Shibagaki N, Ferl RJ, Ehrhardt D, Chong K, Burlingame AL, Wang ZY. An essential role for 14-3-3 proteins in brassinosteroid signal transduction in Arabidopsis. Dev Cell. 2007 Aug;13(2):177-89.

Gao XH, Xiao SL, Yao QF, Wang YJ, Fu XD. An updated GA signaling 'relief of repression' regulatory model. Mol Plant. 2011 Jul;4(4):601-6. Review.

Gietz RD, Schiestl RH, Willems AR, Woods RA. Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. Yeast. 1995 Apr 15;11(4):355-60.

Gingerich DJ, Gagne JM, Salter DW, Hellmann H, Estelle M, Ma L, Vierstra RD. Cullins 3a and 3b assemble with members of the broad complex/tramtrack/bric-a-brac (BTB) protein family to form essential ubiquitin-protein ligases (E3s) in Arabidopsis. J Biol Chem. 2005 May 13;280(19):18810-21.

Giraudat J, Hauge BM, Valon C, Smalle J, Parcy F, Goodman HM. Isolation of the Arabidopsis ABI3 gene by positional cloning. Plant Cell. 1992 Oct;4(10):1251-61.

Gobert A, Park G, Amtmann A, Sanders D, Maathuis FJ. Arabidopsis thaliana cyclic nucleotide gated channel 3 forms a non-selective ion transporter involved in germination and cation transport. J Exp Bot. 2006;57(4):791-800.

Golldack D, Li C, Mohan H, Probst N. Tolerance to drought and salt stress in plants: Unraveling the signaling networks. Front Plant Sci. 2014 Apr 22;5:151. Review.

Gray WM, Ostin A, Sandberg G, Romano CP, Estelle M. High temperature promotes auxin-mediated hypocotyl elongation in Arabidopsis. Proc Natl Acad Sci U S A. 1998 Jun 9;95(12):7197-202.

Grefen C, Harter K. Plant two-component systems: principles, functions, complexity and cross talk. Planta. 2004 Sep;219(5):733-42. Review.

Grefen C, Obrdlik P, Harter K. The determination of protein-protein interactions by the mating-based split-ubiquitin system (mbSUS). Methods Mol Biol. 2009;479:217-33.

Grondin A, Rodrigues O, Verdoucq L, Merlot S, Leonhardt N, Maurel C. Aquaporins Contribute to ABA-Triggered Stomatal Closure through OST1-Mediated Phosphorylation. Plant Cell. 2015 Jul;27(7):1945-54.

Gruhn N, Halawa M, Snel B, Seidl MF, Heyl A. A subfamily of putative cytokinin receptors is revealed by an analysis of the evolution of the two-component signaling system of plants. Plant Physiol. 2014 May;165(1):227-37.

Guilfoyle TJ, Hagen G. Auxin response factors. Curr Opin Plant Biol. 2007 Oct;10(5):453-60. Review.

Guo Y, Xiong L, Song CP, Gong D, Halfter U, Zhu JK. A calcium sensor and its interacting protein kinase are global regulators of abscisic acid signaling in Arabidopsis. Dev Cell. 2002 Aug;3(2):233-44.

Gutierrez R, Lindeboom JJ, Paredez AR, Emons AM, Ehrhardt DW. Arabidopsis cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments. Nat Cell Biol. 2009 Jul;11(7):797-806.

Hagen G, Guilfoyle T. Auxin-responsive gene expression: genes, promoters and regulatory factors. Plant Mol Biol. 2002 Jun-Jul;49(3-4):373-85. Review.

Hager A. Role of the plasma membrane H+-ATPase in auxin-induced elongation growth: historical and new aspects. J Plant Res. 2003 Dec;116(6):483-505.

Halfter U, Ishitani M, Zhu JK. The Arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. Proc Natl Acad Sci U S A. 2000 Mar 28;97(7):3735-40.

Halter T, Imkampe J, Mazzotta S, Wierzba M, Postel S, Bücherl C, Kiefer C, Stahl M, Chinchilla D, Wang X, Nürnberger T, Zipfel C, Clouse S, Borst JW, Boeren S, de Vries SC, Tax F, Kemmerling B. The leucine-rich repeat receptor kinase BIR2 is a negative regulator of BAK1 in plant immunity. Curr Biol. 2014 Jan 20;24(2):134-43.

Hardtke CS, Gohda K, Osterlund MT, Oyama T, Okada K, Deng XW. HY5 stability and activity in arabidopsis is regulated by phosphorylation in its COP1 binding domain. EMBO J. 2000 Sep 15;19(18):4997-5006.

Harrison BR, Masson PH. ARL2, ARG1 and PIN3 define a gravity signal transduction pathway in root statocytes. Plant J. 2008 Jan;53(2):380-92. Epub 2007 Nov 28. PubMed PMID: 18047472.

Haruta M, Burch HL, Nelson RB, Barrett-Wilt G, Kline KG, Mohsin SB, Young JC, Otegui MS, Sussman MR. Molecular characterization of mutant Arabidopsis plants with reduced plasma membrane proton pump activity. J Biol Chem. 2010 Jun 4;285(23):17918-29.

Haruta M, Gray WM, Sussman MR. Regulation of the plasma membrane proton pump (H(+)-ATPase) by phosphorylation. Curr Opin Plant Biol. 2015 Dec;28:68-75. doi: 10.1016/j.pbi.2015.09.005. Epub 2015 Oct 24. Review.

Haruta M, Sabat G, Stecker K, Minkoff BB, Sussman MR. A peptide hormone and its receptor protein kinase regulate plant cell expansion. Science. 2014 Jan 24;343(6169):408-11.

Hass C, Lohrmann J, Albrecht V, Sweere U, Hummel F, Yoo SD, Hwang I, Zhu T, Schäfer E, Kudla J, Harter K. The response regulator 2 mediates ethylene signalling and hormone signal integration in Arabidopsis. EMBO J. 2004 Aug 18;23(16):3290-302.

Haswell ES, Peyronnet R, Barbier-Brygoo H, Meyerowitz EM, Frachisse JM. Two MscS homologs provide mechanosensitive channel activities in the Arabidopsis root. Curr Biol. 2008 May 20;18(10):730-4.

Hayashi Y, Nakamura S, Takemiya A, Takahashi Y, Shimazaki K, Kinoshita T. Biochemical characterization of in vitro phosphorylation and dephosphorylation of the plasma membrane H+-ATPase. Plant Cell Physiol. 2010 Jul;51(7):1186-96.

He JX, Gendron JM, Sun Y, Gampala SS, Gendron N, Sun CQ, Wang ZY. BZR1 is a transcriptional repressor with dual roles in brassinosteroid homeostasis and growth responses. Science. 2005 Mar 11;307(5715):1634-8. Epub 2005 Jan 27. Erratum in: Science. 2005 Jun 17;308(5729):1743

He JX, Gendron JM, Yang Y, Li J, Wang ZY. The GSK3-like kinase BIN2 phosphorylates and destabilizes BZR1, a positive regulator of the brassinosteroid signaling pathway in Arabidopsis. Proc Natl Acad Sci U S A. 2002 Jul 23;99(15):10185-90.

Hellmann H, Estelle M. Plant development: regulation by protein degradation. Science. 2002 Aug 2;297(5582):793-7. Review.

Heyl A, Wulfetange K, Pils B, Nielsen N, Romanov GA, Schmülling T. Evolutionary proteomics identifies amino acids essential for ligand-binding of the cytokinin receptor CHASE domain. BMC Evol Biol. 2007 Apr 17;7:62.

Higuchi M, Pischke MS, Mähönen AP, Miyawaki K, Hashimoto Y, Seki M, Kobayashi M, Shinozaki K, Kato T, Tabata S, Helariutta Y, Sussman MR, Kakimoto T. In planta functions of the Arabidopsis cytokinin receptor family. Proc Natl Acad Sci U S A. 2004 Jun 8;101(23):8821-6.

Hirayama T, Shinozaki K. Perception and transduction of abscisic acid signals: keys to the function of the versatile plant hormone ABA. Trends Plant Sci. 2007 Aug;12(8):343-51. Review.

Hobbie L, Timpte C, Estelle M. Molecular genetics of auxin and cytokinin. Plant Mol Biol. 1994 Dec;26(5):1499-519. Review.

Hoecker U, Quail PH. The phytochrome A-specific signaling intermediate SPA1 interacts directly with COP1, a constitutive repressor of light signaling in Arabidopsis. J Biol Chem. 2001 Oct 12;276(41):38173-8.

Horák J, Janda L, Pekárová B, Hejátko J. Molecular mechanisms of signaling specificity via phosphorelay pathways in Arabidopsis. Curr Protein Pept Sci. 2011 Mar;12(2):126-36. Review.

Hosoda K, Imamura A, Katoh E, Hatta T, Tachiki M, Yamada H, Mizuno T, Yamazaki T. Molecular structure of the GARP family of plant Myb-related DNA binding motifs of the Arabidopsis response regulators. Plant Cell. 2002 Sep;14(9):2015-29.

Hothorn M, Belkhadir Y, Dreux M, Dabi T, Noel JP, Wilson IA, Chory J. Structural basis of steroid hormone perception by the receptor kinase BRI1. Nature. 2011a Jun 12;474(7352):467-71.

Hothorn M, Dabi T, Chory J. Structural basis for cytokinin recognition by Arabidopsis thaliana histidine kinase 4. Nat Chem Biol. 2011b Oct 2;7(11):766-8.

Hsiao TC. Plant responses to water stress. Annual Review of Plant Physiology. 1973 24:519-570

Hu Y, Yu D. BRASSINOSTEROID INSENSITIVE2 interacts with ABSCISIC ACID INSENSITIVE5 to mediate the antagonism of brassinosteroids to abscisic acid during seed germination in Arabidopsis. Plant Cell. 2014 Nov;26(11):4394-408.

Hua J, Sakai H, Nourizadeh S, Chen QG, Bleecker AB, Ecker JR, Meyerowitz EM. EIN4 and ERS2 are members of the putative ethylene receptor gene family in Arabidopsis. Plant Cell. 1998 Aug;10(8):1321-32.

Hua Z, Vierstra RD. The cullin-RING ubiquitin-protein ligases. Annu Rev Plant Biol. 2011;62:299-334. Review.

Huang X, Ouyang X, Yang P, Lau OS, Li G, Li J, Chen H, Deng XW. Arabidopsis FHY3 and HY5 positively mediate induction of COP1 transcription in response to photomorphogenic UV-B light. Plant Cell. 2012 Nov;24(11):4590-606.

Huh SU, Lee SB, Kim HH, Paek KH. ATAF2, a NAC transcription factor, binds to the promoter and regulates NIT2 gene expression involved in auxin biosynthesis. Mol Cells. 2012 Sep;34(3):305-13.

Hutchison CE, Li J, Argueso C, Gonzalez M, Lee E, Lewis MW, Maxwell BB, Perdue TD, Schaller GE, Alonso JM, Ecker JR, Kieber JJ. The Arabidopsis histidine phosphotransfer proteins are redundant positive regulators of cytokinin signaling. Plant Cell. 2006 Nov;18(11):3073-87.

Hwang I, Sheen J. Two-component circuitry in Arabidopsis cytokinin signal transduction. Nature. 2001 Sep 27;413(6854):383-9.

Imaizumi T, Tran HG, Swartz TE, Briggs WR, Kay SA. FKF1 is essential for photoperiodic-specific light signalling in Arabidopsis. Nature. 2003 Nov 20;426(6964):302-6.

Imamura A, Hanaki N, Nakamura A, Suzuki T, Taniguchi M, Kiba T, Ueguchi C, Sugiyama T, Mizuno T. Compilation and characterization of Arabidopsis thaliana response regulators implicated in His-Asp phosphorelay signal transduction. Plant Cell Physiol. 1999 Jul;40(7):733-42.

Ishii T, Matsunaga T, Pellerin P, O'Neill MA, Darvill A, Albersheim P. The plant cell wall polysaccharide rhamnogalacturonan II self-assembles into a covalently cross-linked dimer. J Biol Chem. 1999 May 7;274(19):13098-104.

Ishikawa S, Maekawa M, Arite T, Onishi K, Takamure I, Kyozuka J. Suppression of tiller bud activity in tillering dwarf mutants of rice. Plant Cell Physiol. 2005 Jan;46(1):79-86.

Ito S, Song YH, Imaizumi T. LOV domain-containing F-box proteins: light-dependent protein degradation modules in Arabidopsis. Mol Plant. 2012 May;5(3):573-82. Review.

Jaedicke K, Lichtenthäler AL, Meyberg R, Zeidler M, Hughes J. A phytochrome-phototropin light signaling complex at the plasma membrane. Proc Natl Acad Sci U S A. 2012 Jul 24;109(30):12231-6.

Jaffé FW, Freschet GE, Valdes BM, Runions J, Terry MJ, Williams LE. G protein-coupled receptor-type G proteins are required for light-dependent seedling growth and fertility in Arabidopsis. Plant Cell. 2012 Sep;24(9):3649-68.

Jaillais Y, Hothorn M, Belkhadir Y, Dabi T, Nimchuk ZL, Meyerowitz EM, Chory J. Tyrosine phosphorylation controls brassinosteroid receptor activation by triggering membrane release of its kinase inhibitor. Genes Dev. 2011 Feb 1;25(3):232-7.

Jaillais Y, Vert G. Brassinosteroids, gibberellins and light-mediated signalling are the three-way controls of plant sprouting. Nat Cell Biol. 2012 Aug;14(8):788-90.

James P, Halladay J, Craig EA. Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. Genetics. 1996 Dec;144(4):1425-36.

Jang IC, Henriques R, Seo HS, Nagatani A, Chua NH. Arabidopsis PHYTOCHROME INTERACTING FACTOR proteins promote phytochrome B polyubiquitination by COP1 E3 ligase in the nucleus. Plant Cell. 2010 Jul;22(7):2370-83

Ji Y, Guo H. From endoplasmic reticulum (ER) to nucleus: EIN2 bridges the gap in ethylene signaling. Mol Plant. 2013 Jan;6(1):11-4.

Johansson H, Hughes J. Nuclear phytochrome B regulates leaf flattening through phytochrome interacting factors. Mol Plant. 2014 Nov;7(11):1693-6.

Johnson X, Brcich T, Dun EA, Goussot M, Haurogné K, Beveridge CA, Rameau C. Branching genes are conserved across species. Genes controlling a novel signal in pea are coregulated by other long-distance signals. Plant Physiol. 2006 Nov;142(3):1014-26.

Ju C, Yoon GM, Shemansky JM, Lin DY, Ying ZI, Chang J, Garrett WM, Kessenbrock M, Groth G, Tucker ML, Cooper B, Kieber JJ, Chang C. CTR1 phosphorylates the central regulator EIN2 to control ethylene hormone signaling from the ER membrane to the nucleus in Arabidopsis. Proc Natl Acad Sci U S A. 2012 Nov 20;109(47):19486-91.

Julkowska MM, Hoefsloot HC, Mol S, Feron R, de Boer GJ, Haring MA, Testerink C. Capturing Arabidopsis root architecture dynamics with ROOT-FIT reveals diversity in responses to salinity. Plant Physiol. 2014 Nov;166(3):1387-402.

Kanczewska J, Marco S, Vandermeeren C, Maudoux O, Rigaud JL, Boutry M. Activation of the plant plasma membrane H+-ATPase by phosphorylation and binding of 14-3-3 proteins converts a dimer into a hexamer. Proc Natl Acad Sci U S A. 2005 Aug 16;102(33):11675-80.

Kang B, Wang H, Nam KH, Li J, Li J. Activation-tagged suppressors of a weak brassinosteroid receptor mutant. Mol Plant. 2010 Jan;3(1):260-8.

Kang MY, Yoo SC, Kwon HY, Lee BD, Cho JN, Noh YS, Paek NC. Negative regulatory roles of DE-ETIOLATED1 in flowering time in Arabidopsis. Sci Rep. 2015a May 12;5:9728.

Kang S, Yang F, Li L, Chen H, Chen S, Zhang J. The Arabidopsis transcription factor BRASSINOSTEROID INSENSITIVE1-ETHYL METHANESULFONATE-SUPPRESSOR1 is a direct substrate of MITOGEN-ACTIVATED PROTEIN KINASE6 and regulates immunity. Plant Physiol. 2015b Mar;167(3):1076-86.

Ke J, Ma H, Gu X, Thelen A, Brunzelle JS, Li J, Xu HE, Melcher K. Structural basis for recognition of diverse transcriptional repressors by the TOPLESS family of corepressors. Sci Adv. 2015 Jul 24;1(6):e1500107.

Keinath NF, Waadt R, Brugman R, Schroeder JI, Grossmann G, Schumacher K, Krebs M. Live Cell Imaging with R-GECO1 Sheds Light on flg22- and Chitin-Induced Transient [Ca(2+)]cyt Patterns in Arabidopsis. Mol Plant. 2015 Aug;8(8):1188-200.

Kemmerling B, Schwedt A, Rodriguez P, Mazzotta S, Frank M, Qamar SA, Mengiste T, Betsuyaku S, Parker JE, Müssig C, Thomma BP, Albrecht C, de Vries SC, Hirt H, Nürnberger T. The BRI1-associated kinase 1, BAK1, has a brassinolide-independent role in plant cell-death control. Curr Biol. 2007 Jul 3;17(13):1116-22.

Kendrick RE, Kronenberg GHM. Photomorphogenesis in plants. 1994 Dordrecht, Neth.: Kluwer

Kepinski S, Leyser O. The Arabidopsis F-box protein TIR1 is an auxin receptor. Nature. 2005 May 26;435(7041):446-51.

Kiba T, Aoki K, Sakakibara H, Mizuno T. Arabidopsis response regulator, ARR22, ectopic expression of which results in phenotypes similar to the wol cytokinin-receptor mutant. Plant Cell Physiol. 2004 Aug;45(8):1063-77.

Kieber JJ, Schaller GE. Cytokinins. Arabidopsis Book. 2014 Jan 2;12:e0168.

Kierszniowka, S., Seiwert, B., Schulze, W.X. (2009). Definition of Arabidopsis sterol-rich membrane microdomains by different treatment with methyl-β-cyclodextrin and quantitative proteomics. Mol. Cell. Proteomics 8: 612-623.

Kim B, Jeong YJ, Corvalán C, Fujioka S, Cho S, Park T, Choe S. Darkness and gulliver2/phyB mutation decrease the abundance of phosphorylated BZR1 to activate brassinosteroid signaling in Arabidopsis. Plant J. 2014 Mar;77(5):737-47.

Kim JI, Park JE, Zarate X, Song PS. Phytochrome phosphorylation in plant light signaling. Photochem Photobiol Sci. 2005 Sep;4(9):681-7. Review.

Kim TW, Guan S, Burlingame AL, Wang ZY. The CDG1 kinase mediates brassinosteroid signal transduction from BRI1 receptor kinase to BSU1 phosphatase and GSK3-like kinase BIN2. Mol Cell. 2011 Aug 19;43(4):561-71.

Kim TW, Guan S, Sun Y, Deng Z, Tang W, Shang JX, Sun Y, Burlingame AL, Wang ZY. Brassinosteroid signal transduction from cell-surface receptor kinases to nuclear transcription factors. Nat Cell Biol. 2009 Oct;11(10):1254-60.

Kinoshita T, Caño-Delgado A, Seto H, Hiranuma S, Fujioka S, Yoshida S, Chory J. Binding of brassinosteroids to the extracellular domain of plant receptor kinase BRI1. Nature. 2005 Jan 13;433(7022):167-71.

Kinoshita T, Emi T, Tominaga M, Sakamoto K, Shigenaga A, Doi M, Shimazaki K. Blue-light- and phosphorylation-dependent binding of a 14-3-3 protein to phototropins in stomatal guard cells of broad bean. Plant Physiol. 2003 Dec;133(4):1453-63.

Kinoshita T, Shimazaki K. Analysis of the phosphorylation level in guard-cell plasma membrane H+-ATPase in response to fusicoccin. Plant Cell Physiol. 2001 Apr;42(4):424-32.

Kinoshita T, Shimazaki Ki. Blue light activates the plasma membrane H(+)-ATPase by phosphorylation of the C-terminus in stomatal guard cells. EMBO J. 1999 Oct 15;18(20):5548-58.

Kircher S, Gil P, Kozma-Bognár L, Fejes E, Speth V, Husselstein-Muller T, Bauer D, Adám E, Schäfer E, Nagy F. Nucleocytoplasmic partitioning of the plant photoreceptors phytochrome A, B, C, D, and E is regulated differentially by light and exhibits a diurnal rhythm. Plant Cell. 2002 Jul;14(7):1541-55.

Knight H, Trewavas AJ, Knight MR. Calcium signalling in Arabidopsis thaliana responding to drought and salinity. Plant J. 1997 Nov;12(5):1067-78.

Kobayashi Y, Murata M, Minami H, Yamamoto S, Kagaya Y, Hobo T, Yamamoto A, Hattori T. Abscisic acid-activated SNRK2 protein kinases function in the gene-regulation pathway of ABA signal transduction by phosphorylating ABA response element-binding factors. Plant J. 2005 Dec;44(6):939-49.

Kohchi T, Mukougawa K, Frankenberg N, Masuda M, Yokota A, Lagarias JC. The Arabidopsis HY2 gene encodes phytochromobilin synthase, a ferredoxin- dependent biliverdin reductase. Plant Cell. 2001 Feb;13(2):425-36.

Koncz C. and Schell J. (1986): The promoter of the TL-DNA gene 5 controls the tissuespecific expression of chimeric genes carried by a novel type of Agrobacterium binary vector. Molecular Genetics and Genomics 204, 383–396.

Koornneef M, Reuling G, Karssen C. The isolation and characterization of abscissic acid-insensitive mutants of Arabidopsis thaliana. Physiol. Plant. 1984 61:377-383

Korasick DA, Westfall CS, Lee SG, Nanao MH, Dumas R, Hagen G, Guilfoyle TJ, Jez JM, Strader LC. Molecular basis for AUXIN RESPONSE FACTOR protein interaction and the control of auxin response repression. Proc Natl Acad Sci U S A. 2014 Apr 8;111(14):5427-32.

Koyama H, Toda T, Hara T. Brief exposure to low-pH stress causes irreversible damage to the growing root in Arabidopsis thaliana: pectin-Ca interaction may play an important role in proton rhizotoxicity. J Exp Bot. 2001 Feb;52(355):361-8.

Krzywińska E, Bucholc M, Kulik A, Ciesielski A, Lichocka M, Dębski J, Ludwików A, Dadlez M, Rodriguez PL, Dobrowolska G. Phosphatase ABI1 and okadaic acid-sensitive phosphoprotein phosphatases inhibit salt stress-activated SnRK2.4 kinase. BMC Plant Biol. 2016 Jun 13;16(1):136.

Kuhn JM, Boisson-Dernier A, Dizon MB, Maktabi MH, Schroeder JI. The protein phosphatase AtPP2CA negatively regulates abscisic acid signal transduction in Arabidopsis, and effects of abh1 on AtPP2CA mRNA. Plant Physiol. 2006 Jan;140(1):127-39.

Kumar D. Salicylic acid signaling in disease resistance. Plant Sci. 2014 Nov;228:127-34. Review.

Kumar MN, Jane WN, Verslues PE. Role of the putative osmosensor Arabidopsis histidine kinase1 in dehydration avoidance and low-water-potential response. Plant Physiol. 2013 Feb;161(2):942-53.

Kumari A, Jewaria PK, Bergmann DC, Kakimoto T. Arabidopsis reduces growth under osmotic stress by decreasing SPEECHLESS protein. Plant Cell Physiol. 2014 Dec;55(12):2037-46.

Ladwig F, Dahlke RI, Stührwohldt N, Hartmann J, Harter K, Sauter M. Phytosulfokine Regulates Growth in Arabidopsis through a Response Module at the Plasma Membrane That Includes CYCLIC NUCLEOTIDE-GATED CHANNEL17, H+-ATPase, and BAK1. Plant Cell. 2015 Jun;27(6):1718-29.

Lager I, Andréasson O, Dunbar TL, Andreasson E, Escobar MA, Rasmusson AG. Changes in external pH rapidly alter plant gene expression and modulate auxin and elicitor responses. Plant Cell Environ. 2010 Sep;33(9):1513-28.

Lanzetta PA, Alvarez LJ, Reinach PS, Candia OA. An improved assay for nanomole amounts of inorganic phosphate. Anal Biochem. 1979 Nov 15;100(1):95-7.

Lau OS, Deng XW. The photomorphogenic repressors COP1 and DET1: 20 years later. Trends Plant Sci. 2012 Oct;17(10):584-93. Review.

Laubinger S, Fittinghoff K, Hoecker U. The SPA quartet: a family of WD-repeat proteins with a central role in suppression of photomorphogenesis in arabidopsis. Plant Cell. 2004 Sep;16(9):2293-306.

Laubinger S, Marchal V, Le Gourrierec J, Wenkel S, Adrian J, Jang S, Kulajta C, Braun H, Coupland G, Hoecker U. Arabidopsis SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. Development. 2006 Aug;133(16):3213-22.

Le J, Vandenbussche F, de Cnodder T, van der Straeten D, Verbelen JP. Cell elongation and microtubule behavior in the Arabidopsis hypocotyl: responses to ethylene and auxin. J Plant Growth Regul 2005 24:166–178

Lee CP, Maksaev G, Jensen GS, Murcha MW, Wilson ME, Fricker M, Hell R, Haswell ES, Millar AH, Sweetlove L. MSL1 is a mechanosensitive ion channel that dissipates mitochondrial membrane potential and maintains redox homeostasis in mitochondria during abiotic stress. Plant J. 2016 Aug 9.

Lee JH, Terzaghi W, Gusmaroli G, Charron JB, Yoon HJ, Chen H, He YJ, Xiong Y, Deng XW. Characterization of Arabidopsis and rice DWD proteins and their roles as substrate receptors for CUL4-RING E3 ubiquitin ligases. Plant Cell. 2008 Jan;20(1):152-67.

Lee JH, Yoon HJ, Terzaghi W, Martinez C, Dai M, Li J, Byun MO, Deng XW. DWA1 and DWA2, two Arabidopsis DWD protein components of CUL4-based E3 ligases, act together as negative regulators in ABA signal transduction. Plant Cell. 2010 Jun;22(6):1716-32.

Lee Y, Choi D, Kende H. Expansins: ever-expanding numbers and functions. Curr Opin Plant Biol. 2001 Dec;4(6):527-32.

Leivar P, Monte E, Oka Y, Liu T, Carle C, Castillon A, Huq E, Quail PH. Multiple phytochrome-interacting bHLH transcription factors repress premature seedling photomorphogenesis in darkness. Curr Biol. 2008 Dec 9;18(23):1815-23.

Lemtiri-Chlieh F, MacRobbie EA, Webb AA, Manison NF, Brownlee C, Skepper JN, Chen J, Prestwich GD, Brearley CA. Inositol hexakisphosphate mobilizes an endomembrane store of calcium in guard cells. Proc Natl Acad Sci U S A. 2003 Aug 19;100(17):10091-5

Li J, Chory J. A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell. 1997 Sep 5;90(5):929-38.

Li J, Li G, Gao S, Martinez C, He G, Zhou Z, Huang X, Lee JH, Zhang H, Shen Y, Wang H, Deng XW. Arabidopsis transcription factor ELONGATED HYPOCOTYL5 plays a role in the feedback regulation of phytochrome A signaling. Plant Cell. 2010 Nov;22(11):3634-49.

Li J, Li Y, Chen S, An L. Involvement of brassinosteroid signals in the floral-induction network of Arabidopsis. J Exp Bot. 2010 Oct;61(15):4221-30. Review.

Li J, Nam KH. Regulation of brassinosteroid signaling by a GSK3/SHAGGY-like kinase. Science. 2002a Feb 15;295(5558):1299-301.

Li J, Wen J, Lease KA, Doke JT, Tax FE, Walker JC. BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. Cell. 2002b Jul 26;110(2):213-22.

Li L, Ljung K, Breton G, Schmitz RJ, Pruneda-Paz J, Cowing-Zitron C, Cole BJ, Ivans LJ, Pedmale UV, Jung HS, Ecker JR, Kay SA, Chory J. Linking photoreceptor excitation to changes in plant architecture. Genes Dev. 2012 Apr 15;26(8):785-90.

Li R, Wang J, Li S, Zhang L, Qi C, Weeda S, Zhao B, Ren S, Guo YD. Plasma Membrane Intrinsic Proteins SlPIP2;1, SlPIP2;7 and SlPIP2;5 Conferring Enhanced Drought Stress Tolerance in Tomato. Sci Rep. 2016a Aug 22;6:31814.

Li SB, Xie ZZ, Hu CG, Zhang JZ. A Review of Auxin Response Factors (ARFs) in Plants. Front Plant Sci. 2016b Feb 3;7:47. Review.

Lian HL, He SB, Zhang YC, Zhu DM, Zhang JY, Jia KP, Sun SX, Li L, Yang HQ. Blue-light-dependent interaction of cryptochrome 1 with SPA1 defines a dynamic signaling mechanism. Genes Dev. 2011 May 15;25(10):1023-8.

Lin C, Robertson DE, Ahmad M, Raibekas AA, Jorns MS, Dutton PL, Cashmore AR. Association of flavin adenine dinucleotide with the Arabidopsis blue light receptor CRY1. Science. 1995 Aug 18;269(5226):968-70.

Lin C. Blue light receptors and signal transduction. Plant Cell. 2002;14 Suppl:S207-25. Review.

Lin LL, Hsu CL, Hu CW, Ko SY, Hsieh HL, Huang HC, Juan HF. Integrating Phosphoproteomics and Bioinformatics to Study Brassinosteroid-Regulated Phosphorylation Dynamics in Arabidopsis. BMC Genomics. 2015 Jul 18;16:533.

Lin R, Teng Y, Park HJ, Ding L, Black C, Fang P, Wang H. Discrete and essential roles of the multiple domains of Arabidopsis FHY3 in mediating phytochrome A signal transduction. Plant Physiol. 2008 Oct;148(2):981-92.

Lippold F, Sanchez DH, Musialak M, Schlereth A, Scheible WR, Hincha DK, Udvardi MK. AtMyb41 regulates transcriptional and metabolic responses to osmotic stress in Arabidopsis. Plant Physiol. 2009 Apr;149(4):1761-72.

Liscum E, Hodgson DW, Campbell TJ. Blue light signaling through the cryptochromes and phototropins. So that's what the blues is all about. Plant Physiol. 2003 Dec;133(4):1429-36. Review.

Liscum E, Reed JW. Genetics of Aux/IAA and ARF action in plant growth and development. Plant Mol Biol. 2002 Jun-Jul;49(3-4):387-400. Review.

Litchfield DW. Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. Biochem J. 2003 Jan 1;369(Pt 1):1-15. Review.

Liu B, Zuo Z, Liu H, Liu X, Lin C. Arabidopsis cryptochrome 1 interacts with SPA1 to suppress COP1 activity in response to blue light. Genes Dev. 2011 May 15;25(10):1029-34.

Liu D, Bienkowska J, Petosa C, Collier RJ, Fu H, Liddington R. Crystal structure of the zeta isoform of the 14-3-3 protein. Nature. 1995 Jul 13;376(6536):191-4.

Liu J, Elmore JM, Fuglsang AT, Palmgren MG, Staskawicz BJ, Coaker G. RIN4 functions with plasma membrane H+-ATPases to regulate stomatal apertures during pathogen attack. PLoS Biol. 2009 Jun 30;7(6):e1000139.

Liu J, Ishitani M, Halfter U, Kim CS, Zhu JK. The Arabidopsis thaliana SOS2 gene encodes a protein kinase that is required for salt tolerance. Proc Natl Acad Sci U S A. 2000 Mar 28;97(7):3730-4.

Liu J, Zhu JK. An Arabidopsis mutant that requires increased calcium for potassium nutrition and salt tolerance. Proc Natl Acad Sci U S A. 1997 Dec 23;94(26):14960-4.

Liu Q, Xu C, Wen CK. Genetic and transformation studies reveal negative regulation of ERS1 ethylene receptor signaling in Arabidopsis. BMC Plant Biol. 2010 Apr 8;10:60.

Lloyd C, Chan J. The parallel lives of microtubules and cellulose microfibrils. Curr Opin Plant Biol. 2008 Dec;11(6):641-6. Review.

Lohrmann J, Harter K. Plant two-component signaling systems and the role of response regulators. Plant Physiol. 2002 Feb;128(2):363-9. Review.

Lohrmann J, Sweere U, Zabaleta E, Bäurle I, Keitel C, Kozma-Bognar L, Brennicke A, Schäfer E, Kudla J, Harter K. The response regulator ARR2: a pollen-specific transcription factor involved in the expression of nuclear genes for components of mitochondrial complex I in Arabidopsis. Mol Genet Genomics. 2001 Mar;265(1):2-13.

Lopez-Molina L, Mongrand S, Chua NH. A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in Arabidopsis. Proc Natl Acad Sci U S A. 2001 Apr 10;98(8):4782-7.

Lorenz, A. Die Funktion der AHK1 bei der Regulation des Zytoskeletts durch Interaktion mit Zytoskelett-assoziierten Proteinen. bachelorthesis. 2014

Lu XD, Zhou CM, Xu PB, Luo Q, Lian HL, Yang HQ. Red-light-dependent interaction of phyB with SPA1 promotes COP1-SPA1 dissociation and photomorphogenic development in Arabidopsis. Mol Plant. 2015 Mar;8(3):467-78.

Luo XM, Lin WH, Zhu S, Zhu JY, Sun Y, Fan XY, Cheng M, Hao Y, Oh E, Tian M, Liu L, Zhang M, Xie Q, Chong K, Wang ZY. Integration of light- and brassinosteroid-signaling pathways by a GATA transcription factor in Arabidopsis. Dev Cell. 2010 Dec 14;19(6):872-83.

Lynch T, Erickson BJ, Finkelstein RR. Direct interactions of ABA-insensitive(ABI)-clade protein phosphatase(PP)2Cs with calcium-dependent protein kinases and ABA response element-binding bZIPs may contribute to turning off ABA response. Plant Mol Biol. 2012 Dec;80(6):647-58.

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E. Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science. 2009 May 22;324(5930):1064-8.

Maeda T, Wurgler-Murphy SM, Saito H. A two-component system that regulates an osmosensing MAP kinase cascade in yeast. Nature. 1994 May 19;369(6477):242-5.

Maksaev G, Haswell ES. MscS-Like10 is a stretch-activated ion channel from Arabidopsis thaliana with a preference for anions. Proc Natl Acad Sci U S A. 2012 Nov 13;109(46):19015-20.

Manak MS, Ferl RJ. Divalent cation effects on interactions between multiple Arabidopsis 14-3-3 isoforms and phosphopeptide targets. Biochemistry. 2007 Jan 30;46(4):1055-63.

Mandava NB. Plant growth-promoting brassinosteroids. Ann. Rev. Plant Physiol. Plant Mol. Bio. 1988; 39:23-52

Marhavý P, Vanstraelen M, De Rybel B, Zhaojun D, Bennett MJ, Beeckman T, Benková E. Auxin reflux between the endodermis and pericycle promotes lateral root initiation. EMBO J. 2013 Jan 9;32(1):149-58.

Mason MG, Li J, Mathews DE, Kieber JJ, Schaller GE. Type-B response regulators display overlapping expression patterns in Arabidopsis. Plant Physiol. 2004 Jun;135(2):927-37.

Maurel C, Boursiac Y, Luu DT, Santoni V, Shahzad Z, Verdoucq L. Aquaporins in Plants. Physiol Rev. 2015 Oct;95(4):1321-58. Review.

McGinnis KM, Thomas SG, Soule JD, Strader LC, Zale JM, Sun TP, Steber CM. The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell. 2003 May;15(5):1120-30.

Medzihradszky M, Bindics J, Ádám É, Viczián A, Klement É, Lorrain S, Gyula P, Mérai Z, Fankhauser C, Medzihradszky KF, Kunkel T, Schäfer E, Nagy F. Phosphorylation of phytochrome B inhibits light-induced signaling via accelerated dark reversion in Arabidopsis. Plant Cell. 2013 Feb;25(2):535-44.

Menon C, Sheerin DJ, Hiltbrunner A. SPA proteins: SPAnning the gap between visible light and gene expression. Planta. 2016 Aug;244(2):297-312. Review.

Merchante C, Alonso JM, Stepanova AN. Ethylene signaling: simple ligand, complex regulation. Curr Opin Plant Biol. 2013 Oct;16(5):554-60.

Mira-Rodado V, Sweere U, Grefen C, Kunkel T, Fejes E, Nagy F, Schäfer E, Harter K. Functional cross-talk between two-component and phytochrome B signal transduction in Arabidopsis. J Exp Bot. 2007;58(10):2595-607.

Miura K, Lee J, Jin JB, Yoo CY, Miura T, Hasegawa PM. Sumoylation of ABI5 by the Arabidopsis SUMO E3 ligase SIZ1 negatively regulates abscisic acid signaling. Proc Natl Acad Sci U S A. 2009 Mar 31;106(13):5418-23.

Miwa K, Ishikawa K, Terada K, Yamada H, Suzuki T, Yamashino T, Mizuno T. Identification of amino acid substitutions that render the Arabidopsis cytokinin receptor histidine kinase AHK4 constitutively active. Plant Cell Physiol. 2007 Dec;48(12):1809-14.

Mizoguchi T, Irie K, Hirayama T, Hayashida N, Yamaguchi-Shinozaki K, Matsumoto K, Shinozaki K. A gene encoding a mitogen-activated protein kinase kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in Arabidopsis thaliana. Proc Natl Acad Sci U S A. 1996 Jan 23;93(2):765-9.

Młodzińska E, Kłobus G, Christensen MD, Fuglsang AT. The plasma membrane H(+)-ATPase AHA2 contributes to the root architecture in response to different nitrogen supply. Physiol Plant. 2015 Jun;154(2):270-82.

Mockaitis K, Estelle M. Auxin receptors and plant development: a new signaling paradigm. Annu Rev Cell Dev Biol. 2008;24:55-80. Review.

Moeller HB, Slengerik-Hansen J, Aroankins T, Assentoft M, MacAulay N, Moestrup SK, Bhalla V, Fenton RA. Regulation of the Water Channel Aquaporin-2 via 14-3-3θ and -ζ. J Biol Chem. 2016 Jan 29;291(5):2469-84.

Monshausen GB, Bibikova TN, Weisenseel MH, Gilroy S. Ca2+ regulates reactive oxygen species production and pH during mechanosensing in Arabidopsis roots. Plant Cell. 2009 Aug;21(8):2341-56.

Monshausen GB, Gilroy S. Feeling green: mechanosensing in plants. Trends Cell Biol. 2009 May;19(5):228-35. Review.

Mora-García S, Vert G, Yin Y, Caño-Delgado A, Cheong H, Chory J. Nuclear protein phosphatases with Kelch-repeat domains modulate the response to brassinosteroids in Arabidopsis. Genes Dev. 2004 Feb 15;18(4):448-60.

Mosher S, Seybold H, Rodriguez P, Stahl M, Davies KA, Dayaratne S, Morillo SA, Wierzba M, Favery B, Keller H, Tax FE, Kemmerling B. The tyrosine-sulfated peptide receptors PSKR1 and PSY1R

modify the immunity of Arabidopsis to biotrophic and necrotrophic pathogens in an antagonistic manner. Plant J. 2013 Feb;73(3):469-82.

Mudgil Y, Shiu SH, Stone SL, Salt JN, Goring DR. A large complement of the predicted Arabidopsis ARM repeat proteins are members of the U-box E3 ubiquitin ligase family. Plant Physiol. 2004 Jan;134(1):59-66. Epub 2003 Dec 4.

Mulekar JJ, Huq E. Expanding roles of protein kinase CK2 in regulating plant growth and development. J Exp Bot. 2014 Jun;65(11):2883-93. Review.

Müller B, Sheen J. Arabidopsis cytokinin signaling pathway. Sci STKE. 2007 Oct 9;2007(407):cm5. Review.

Murase K, Hirano Y, Sun TP, Hakoshima T. Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. Nature. 2008 Nov 27;456(7221):459-63.

Mutasa-Gottgens E, Qi A, Mathews A, Thomas S, Phillips A, Hedden P. Modification of gibberellin signalling (metabolism & signal transduction) in sugar beet: analysis of potential targets for crop improvement. Transgenic Res. 2009 Apr;18(2):301-8.

Nam KH, Li J. BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell. 2002 Jul 26;110(2):203-12.

Neff MM, Fankhauser C, Chory J. Light: an indicator of time and place. Genes Dev. 2000 Feb 1;14(3):257-71. Review.

Neff MM, Nguyen SM, Malancharuvil EJ, Fujioka S, Noguchi T, Seto H, Tsubuki M, Honda T, Takatsuto S, Yoshida S, Chory J. BAS1: A gene regulating brassinosteroid levels and light responsiveness in Arabidopsis. Proc Natl Acad Sci U S A. 1999 Dec 21;96(26):15316-23.

Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT. Hydrogen peroxide and nitric oxide as signalling molecules in plants. J Exp Bot. 2002 May;53(372):1237-47. Review.

Nelson BK, Cai X, Nebenführ A. A multicolored set of in vivo organelle markers for co-localization studies in Arabidopsis and other plants. Plant J. 2007 Sep;51(6):1126-36.

Nemhauser JL, Hong F, Chory J. Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. Cell. 2006 Aug 11;126(3):467-75.

Newton RP, Roef L, Witters E, van Onckelen H. New Phytol. 1999;143:427-455. Review.

Nguyen KH, Ha CV, Nishiyama R, Watanabe Y, Leyva-González MA, Fujita Y, Tran UT, Li W, Tanaka M, Seki M, Schaller GE, Herrera-Estrella L, Tran LS. Arabidopsis type B cytokinin response regulators ARR1, ARR10, and ARR12 negatively regulate plant responses to drought. Proc Natl Acad Sci U S A. 2016 Mar 15;113(11):3090-5.

Nishimura N, Sarkeshik A, Nito K, Park SY, Wang A, Carvalho PC, Lee S, Caddell DF, Cutler SR, Chory J, Yates JR, Schroeder JI. PYR/PYL/RCAR family members are major in-vivo ABI1 protein phosphatase 2C-interacting proteins in Arabidopsis. Plant J. 2010 Jan;61(2):290-9.

Nishimura N, Yoshida T, Kitahata N, Asami T, Shinozaki K, Hirayama T. ABA-Hypersensitive Germination1 encodes a protein phosphatase 2C, an essential component of abscisic acid signaling in Arabidopsis seed. Plant J. 2007 Jun;50(6):935-49.

Nixdorf M, Hoecker U. SPA1 and DET1 act together to control photomorphogenesis throughout plant development. Planta. 2010 Mar;231(4):825-33.

Noguchi T, Fujioka S, Choe S, Takatsuto S, Yoshida S, Yuan H, Feldmann KA, Tax FE. Brassinosteroid-insensitive dwarf mutants of Arabidopsis accumulate brassinosteroids. Plant Physiol. 1999 Nov;121(3):743-52.

Nühse TS, Bottrill AR, Jones AM, Peck SC. Quantitative phosphoproteomic analysis of plasma membrane proteins reveals regulatory mechanisms of plant innate immune responses. Plant J. 2007 Sep;51(5):931-40.

Nühse TS, Peck SC, Hirt H, Boller T. Microbial elicitors induce activation and dual phosphorylation of the Arabidopsis thaliana MAPK 6. J Biol Chem. 2000 Mar 17;275(11):7521-6.

Odell JT, Nagy F, Chua NH. Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature. 1985 Feb 28-Mar 6;313(6005):810-2.

Oh E, Yamaguchi S, Kamiya Y, Bae G, Chung WI, Choi G. Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in Arabidopsis. Plant J. 2006 Jul;47(1):124-39.

Oh E, Zhu JY, Ryu H, Hwang I, Wang ZY. TOPLESS mediates brassinosteroid-induced transcriptional repression through interaction with BZR1. Nat Commun. 2014 Jun 18;5:4140.

Oh E, Zhu JY, Wang ZY. Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. Nat Cell Biol. 2012 Aug;14(8):802-9.

Oka A, Sakai H, Iwakoshi S. His-Asp phosphorelay signal transduction in higher plants: receptors and response regulators for cytokinin signaling in Arabidopsis thaliana. Genes Genet Syst. 2002 Dec;77(6):383-91. Review.

Osakabe Y, Arinaga N, Umezawa T, Katsura S, Nagamachi K, Tanaka H, Ohiraki H, Yamada K, Seo SU, Abo M, Yoshimura E, Shinozaki K, Yamaguchi-Shinozaki K. Osmotic stress responses and plant growth controlled by potassium transporters in Arabidopsis. Plant Cell. 2013 Feb;25(2):609-24.

Osterlund MT, Hardtke CS, Wei N, Deng XW. Targeted destabilization of HY5 during light-regulated development of Arabidopsis. Nature. 2000 May 25;405(6785):462-6.

Ottmann C, Marco S, Jaspert N, Marcon C, Schauer N, Weyand M, Vandermeeren C, Duby G, Boutry M, Wittinghofer A, Rigaud JL, Oecking C. Structure of a 14-3-3 coordinated hexamer of the plant plasma membrane H+ -ATPase by combining X-ray crystallography and electron cryomicroscopy. Mol Cell. 2007 Feb 9;25(3):427-40.

Oyama T, Shimura Y, Okada K. The Arabidopsis HY5 gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. Genes Dev. 1997 Nov 15;11(22):2983-95.

Paál A. Über phototropische Reizleitung. Jahrb. wiss. Bot. 1918; 58: 406

Pacín M, Legris M, Casal JJ. Rapid decline in nuclear constitutive photomorphogenesis1 abundance anticipates the stabilization of its target elongated hypocotyl5 in the light. Plant Physiol. 2014 Mar;164(3):1134-8.

Palmgren MG. PLANT PLASMA MEMBRANE H+-ATPases: Powerhouses for Nutrient Uptake. Annu Rev Plant Physiol Plant Mol Biol. 2001 Jun;52:817-845.

Pandey S, Nelson DC, Assmann SM. Two novel GPCR-type G proteins are abscisic acid receptors in Arabidopsis. Cell. 2009 Jan 9;136(1):136-48.

Park E, Park J, Kim J, Nagatani A, Lagarias JC, Choi G. Phytochrome B inhibits binding of phytochrome-interacting factors to their target promoters. Plant J. 2012 Nov;72(4):537-46.

Park J, Nguyen KT, Park E, Jeon JS, Choi G. DELLA proteins and their interacting RING Finger proteins repress gibberellin responses by binding to the promoters of a subset of gibberellin-responsive genes in Arabidopsis. Plant Cell. 2013 Mar;25(3):927-43.

Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow TF, Alfred SE, Bonetta D, Finkelstein R, Provart NJ, Desveaux D, Rodriguez PL, McCourt P, Zhu JK, Schroeder JI, Volkman BF, Cutler SR. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. Science. 2009 May 22;324(5930):1068-71.

Pekárová B, Klumpler T, Třísková O, Horák J, Jansen S, Dopitová R, Borkovcová P, Papoušková V, Nejedlá E, Sklenář V, Marek J, Zídek L, Hejátko J, Janda L. Structure and binding specificity of the receiver domain of sensor histidine kinase CKI1 from Arabidopsis thaliana. Plant J. 2011 Sep;67(5):827-39.

Pekárová B, Szmitkowska A, Dopitová R, Degtjarik O, Žídek L, Hejátko J. Structural Aspects of Multistep Phosphorelay-Mediated Signaling in Plants. Mol Plant. 2016 Jan 4;9(1):71-85. Review.

Peng H, Zhao J, Neff MM. ATAF2 integrates Arabidopsis brassinosteroid inactivation and seedling photomorphogenesis. Development. 2015 Dec 1;142(23):4129-38.

Péret B, Li G, Zhao J, Band LR, Voß U, Postaire O, Luu DT, Da Ines O, Casimiro I, Lucas M, Wells DM, Lazzerini L, Nacry P, King JR, Jensen OE, Schäffner AR, Maurel C, Bennett MJ. Auxin regulates aquaporin function to facilitate lateral root emergence. Nat Cell Biol. 2012 Oct;14(10):991-8.

Petroski MD, Deshaies RJ. Function and regulation of cullin-RING ubiquitin ligases. Nat Rev Mol Cell Biol. 2005 Jan;6(1):9-20. Review.

Pham J, Desikan R. Modulation of ROS production and hormone levels by AHK5 during abiotic and biotic stress signaling. Plant Signal Behav. 2012 Aug;7(8):893-7.

Phee BK, Kim JI, Shin DH, Yoo J, Park KJ, Han YJ, Kwon YK, Cho MH, Jeon JS, Bhoo SH, Hahn TR. A novel protein phosphatase indirectly regulates phytochrome-interacting factor 3 via phytochrome. Biochem J. 2008 Oct 15;415(2):247-55.

Polko JK, van Zanten M, van Rooij JA, Marée AF, Voesenek LA, Peeters AJ, Pierik R. Ethylene-induced differential petiole growth in Arabidopsis thaliana involves local microtubule reorientation and cell expansion. New Phytol. 2012 Jan;193(2):339-48.

Posas F, Wurgler-Murphy SM, Maeda T, Witten EA, Thai TC, Saito H. Yeast HOG1 MAP kinase cascade is regulated by a multistep phosphorelay mechanism in the SLN1-YPD1-SSK1 "two-component" osmosensor. Cell. 1996 Sep 20;86(6):865-75.

Prak S, Hem S, Boudet J, Viennois G, Sommerer N, Rossignol M, Maurel C, Santoni V. Multiple phosphorylations in the C-terminal tail of plant plasma membrane aquaporins: role in subcellular trafficking of AtPIP2;1 in response to salt stress. Mol Cell Proteomics. 2008 Jun;7(6):1019-30.

Punwani JA, Hutchison CE, Schaller GE, Kieber JJ. The subcellular distribution of the Arabidopsis histidine phosphotransfer proteins is independent of cytokinin signaling. Plant J. 2010 May;62(3):473-82.

Quail PP. An emerging molecular map of the phytochromes. Plant Cell Environ. 1997 20:657-66

Rayle DL, Cleland R. Control of plant cell enlargement by hydrogen ions. Curr Top Dev Biol. 1977;11:187-214. Review.

Rayle DL, Cleland RE. The Acid Growth Theory of auxin-induced cell elongation is alive and well. Plant Physiol. 1992 Aug;99(4):1271-4.

Reiser V, Raitt DC, Saito H. Yeast osmosensor Sln1 and plant cytokinin receptor Cre1 respond to changes in turgor pressure. J Cell Biol. 2003 Jun 23;161(6):1035-40.

Ren C, Zhu X, Zhang P, Gong Q. Arabidopsis COP1-interacting protein 1 is a positive regulator of ABA response. Biochem Biophys Res Commun. 2016 Sep 2;477(4):847-53.

Ridley BL, O'Neill MA, Mohnen D. Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. Phytochemistry. 2001 Jul;57(6):929- 7. Review.

Riechmann JL, Heard J, Martin G, Reuber L, Jiang C, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, Creelman R, Pilgrim M, Broun P, Zhang JZ, Ghandehari D, Sherman BK, Yu G. Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. Science. 2000 Dec 15;290(5499):2105-10.

Rinaldi MA, Liu J, Enders TA, Bartel B, Strader LC. A gain-of-function mutation in IAA16 confers reduced responses to auxin and abscisic acid and impedes plant growth and fertility. Plant Mol Biol. 2012 Jul;79(4-5):359- 73.

Rivas-San Vicente M, Plasencia J. Salicylic acid beyond defence: its role in plant growth and development. J Exp Bot. 2011 Jun;62(10):3321-38. Review.

Rolauffs S, Fackendahl P, Sahm J, Fiene G, Hoecker U. Arabidopsis COP1 and SPA genes are essential for plant elongation but not for acceleration of flowering time in response to a low red light to far-red light ratio. Plant Physiol. 2012 Dec;160(4):2015-27.

Rowe JH, Topping JF, Liu J, Lindsey K. Abscisic acid regulates root growth under osmotic stress conditions via an interacting hormonal network with cytokinin, ethylene and auxin. New Phytol. 2016 Jul;211(1):225-39.

Rudashevskaya EL, Ye J, Jensen ON, Fuglsang AT, Palmgren MG. Phosphosite mapping of P-type plasma membrane H+-ATPase in homologous and heterologous environments. J Biol Chem. 2012 Feb 10;287(7):4904-13.

Ruegger M, Dewey E, Gray WM, Hobbie L, Turner J, Estelle M. The TIR1 protein of Arabidopsis functions in auxin response and is related to human SKP2 and yeast grr1p. Genes Dev. 1998 Jan 15;12(2):198-207.

Ryu H, Cho H, Bae W, Hwang I. Control of early seedling development by BES1/TPL/HDA19-mediated epigenetic regulation of ABI3. Nat Commun. 2014 Jun 18;5:4138.

Saijo Y, Zhu D, Li J, Rubio V, Zhou Z, Shen Y, Hoecker U, Wang H, Deng XW. Arabidopsis COP1/SPA1 complex and FHY1/FHY3 associate with distinct phosphorylated forms of phytochrome A in balancing light signaling. Mol Cell. 2008 Aug 22;31(4):607-13.

Saito H, Tatebayashi K. Regulation of the osmoregulatory HOG MAPK cascade in yeast. J Biochem. 2004 Sep;136(3):267-72. Review.

Sakai H, Honma T, Aoyama T, Sato S, Kato T, Tabata S, Oka A. ARR1, a transcription factor for genes immediately responsive to cytokinins. Science. 2001 Nov 16;294(5546):1519-21.

Sakai H, Hua J, Chen QG, Chang C, Medrano LJ, Bleecker AB, Meyerowitz EM. ETR2 is an ETR1-like gene involved in ethylene signaling in Arabidopsis. Proc Natl Acad Sci U S A. 1998 May 12;95(10):5812-7.

Salinas P, Fuentes D, Vidal E, Jordana X, Echeverria M, Holuigue L. An extensive survey of CK2 alpha and beta subunits in Arabidopsis: multiple isoforms exhibit differential subcellular localization. Plant Cell Physiol. 2006 Sep;47(9):1295-308.

Salomé PA, To JP, Kieber JJ, McClung CR. Arabidopsis response regulators ARR3 and ARR4 play cytokinin-independent roles in the control of circadian period. Plant Cell. 2006 Jan;18(1):55-69.

Sambrook, J., Fritsch, E.F. and Maniatis, T.; Hrsg. 1989. Molecular cloning – A Laboratory Manual, 2nd Edition. Cold Spring Harbour Laboratory Press, New York

Sambrook, Joseph; Russell, David W (David William); Hrsg. (2001): Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, c2001. NLM ID: 100963935

Sancar A. Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. Chem Rev. 2003 Jun;103(6):2203-37. Review.

Sanders DA, Gillece-Castro BL, Stock AM, Burlingame AL, Koshland DE Jr. Identification of the site of phosphorylation of the chemotaxis response regulator protein, CheY. J Biol Chem. 1989 Dec 25;264(36):21770-8.

Sasaki A, Itoh H, Gomi K, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Jeong DH, An G, Kitano H, Ashikari M, Matsuoka M. Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. Science. 2003 Mar 21;299(5614):1896-8.

Sato A, Sato Y, Fukao Y, Fujiwara M, Umezawa T, Shinozaki K, Hibi T, Taniguchi M, Miyake H, Goto DB, Uozumi N. Threonine at position 306 of the KAT1 potassium channel is essential for channel activity and is a target site for ABA-activated SnRK2/OST1/SnRK2.6 protein kinase. Biochem J. 2009 Dec 10;424(3):439-48.

Schaller GE, Doi K, Hwang I, Kieber JJ, Khurana JP, Kurata N, Mizuno T, Pareek A, Shiu SH, Wu P, Yip WK. Nomenclature for two-component signaling elements of rice. Plant Physiol. 2007 Feb;143(2):555-7.

Schaller GE, Kieber JJ, Shiu SH. Two-component signaling elements and histidyl-aspartyl phosphorelays. Arabidopsis Book. 2008;6:e0112.

Schiele, L. Interaktionsstudien der Caseinkinase2-Untereinheiten und Isoformen der Protonenpumpen AHA1 und AHA2 aus Arabidopsis thaliana. Masterarbeit. 2016

Schliess F, Reinehr R, Häussinger D. Osmosensing and signaling in the regulation of mammalian cell function. FEBS J. 2007 Nov;274(22):5799-803.

Schweighofer A, Kazanaviciute V, Scheikl E, Teige M, Doczi R, Hirt H, Schwanninger M, Kant M, Schuurink R, Mauch F, Buchala A, Cardinale F, Meskiene I. The PP2C-type phosphatase AP2C1, which negatively regulates MPK4 and MPK6, modulates innate immunity, jasmonic acid, and ethylene levels in Arabidopsis. Plant Cell. 2007 Jul;19(7):2213-24.

Sedbrook JC, Chen R, Masson PH. ARG1 (altered response to gravity) encodes a DnaJ-like protein that potentially interacts with the cytoskeleton. Proc Natl Acad Sci U S A. 1999 Feb 2;96(3):1140-5.

Seo HS, Watanabe E, Tokutomi S, Nagatani A, Chua NH. Photoreceptor ubiquitination by COP1 E3 ligase desensitizes phytochrome A signaling. Genes Dev. 2004 Mar 15;18(6):617-22.

Seto Y, Yamaguchi S. Strigolactone biosynthesis and perception. Curr Opin Plant Biol. 2014 Oct;21:1-6. Review.

Shang Y, Dai C, Lee MM, Kwak JM, Nam KH. BRI1-Associated Receptor Kinase 1 Regulates Guard Cell ABA Signaling Mediated by Open Stomata 1 in Arabidopsis. Mol Plant. 2016 Mar 7;9(3):447-60.

Shapiguzov A, Lyukevich AA, Allakhverdiev SI, Sergeyenko TV, Suzuki I, Murata N, Los DA. Osmotic shrinkage of cells of Synechocystis sp. PCC 6803 by water efflux via aquaporins regulates osmostress-inducible gene expression. Microbiology. 2005 Feb;151(Pt 2):447-55.

Sharrock RA, Clack T. Heterodimerization of type II phytochromes in Arabidopsis. Proc Natl Acad Sci U S A. 2004 Aug 3;101(31):11500-5.

She J, Han Z, Kim TW, Wang J, Cheng W, Chang J, Shi S, Wang J, Yang M, Wang ZY, Chai J. Structural insight into brassinosteroid perception by BRI1. Nature. 2011 Jun 12;474(7352):472-6.

Sheerin DJ, Menon C, zur Oven-Krockhaus S, Enderle B, Zhu L, Johnen P, Schleifenbaum F, Stierhof YD, Huq E, Hiltbrunner A. Light-activated phytochrome A and B interact with members of the SPA family to promote photomorphogenesis in Arabidopsis by reorganizing the COP1/SPA complex. Plant Cell. 2015 Jan;27(1):189-201.

Shen H, Moon J, Huq E. PIF1 is regulated by light-mediated degradation through the ubiquitin-26S proteasome pathway to optimize photomorphogenesis of seedlings in Arabidopsis. Plant J. 2005 Dec;44(6):1023-35.

Shen YY, Wang XF, Wu FQ, Du SY, Cao Z, Shang Y, Wang XL, Peng CC, Yu XC, Zhu SY, Fan RC, Xu YH, Zhang DP. The Mg-chelatase H subunit is an abscisic acid receptor. Nature. 2006 Oct 19;443(7113):823-6.

Shibaoka H. Regulation by gibberellins of the orientation of cortical microtubules in plant-cells. Aust. J. Plant Physiol., 1993, 20, 461-70

Shimada A, Ueguchi-Tanaka M, Nakatsu T, Nakajima M, Naoe Y, Ohmiya H, Kato H, Matsuoka M. Structural basis for gibberellin recognition by its receptor GID1. Nature. 2008 Nov 27;456(7221):520-3.

Shimomura O, Johnson FH, Saiga Y. Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. J Cell Comp Physiol. 1962 Jun;59:223-39.

Siddiqui H, Khan S, Rhodes BM, Devlin PF. FHY3 and FAR1 Act Downstream of Light Stable Phytochromes. Front Plant Sci. 2016 Feb 22;7:175.

Sjövall-Larsen S, Alexandersson E, Johansson I, Karlsson M, Johanson U, Kjellbom P. Purification and characterization of two protein kinases acting on the aquaporin SoPIP2;1. Biochim Biophys Acta. 2006 Aug;1758(8):1157-64.

Skerker JM, Perchuk BS, Siryaporn A, Lubin EA, Ashenberg O, Goulian M, Laub MT. Rewiring the specificity of two-component signal transduction systems. Cell. 2008 Jun 13;133(6):1043-54.

Smalle J, Vierstra RD. The ubiquitin 26S proteasome proteolytic pathway. Annu Rev Plant Biol. 2004;55:555-90. Review.

Smith SM, Li J. Signalling and responses to strigolactones and karrikins. Curr Opin Plant Biol. 2014 Oct;21:23-9. Review.

Smith TF, Gaitatzes C, Saxena K, Neer EJ. The WD repeat: a common architecture for diverse functions. Trends Biochem Sci. 1999 May;24(5):181-5. Review.

Solano R, Stepanova A, Chao Q, Ecker JR. Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev. 1998 Dec 1;12(23):3703-14.

Soon FF, Ng LM, Zhou XE, West GM, Kovach A, Tan MH, Suino-Powell KM, He Y, Xu Y, Chalmers MJ, Brunzelle JS, Zhang H, Yang H, Jiang H, Li J, Yong EL, Cutler S, Zhu JK, Griffin PR, Melcher K, Xu HE. Molecular mimicry regulates ABA signaling by SnRK2 kinases and PP2C phosphatases. Science. 2012 Jan 6;335(6064):85-8.

Spartz AK, Ren H, Park MY, Grandt KN, Lee SH, Murphy AS, Sussman MR, Overvoorde PJ, Gray WM. SAUR Inhibition of PP2C-D Phosphatases Activates Plasma Membrane H+-ATPases to Promote Cell Expansion in Arabidopsis. Plant Cell. 2014 May 23;26(5):2129-2142.

Sreeramulu S, Mostizky Y, Sunitha S, Shani E, Nahum H, Salomon D, Hayun LB, Gruetter C, Rauh D, Ori N, Sessa G. BSKs are partially redundant positive regulators of brassinosteroid signaling in Arabidopsis. Plant J. 2013 Jun;74(6):905-19.

Stavang JA, Gallego-Bartolomé J, Gómez MD, Yoshida S, Asami T, Olsen JE, García-Martínez JL, Alabadí D, Blázquez MA. Hormonal regulation of temperature-induced growth in Arabidopsis. Plant J. 2009 Nov;60(4):589-601.

Steen DA, Chadwick AV. Ethylene Effects in Pea Stem Tissue: Evidence of microtubule mediation. Plant Physiol. 1981 Mar;67(3):460-6.

Stepanova AN, Yun J, Robles LM, Novak O, He W, Guo H, Ljung K, Alonso JM. The Arabidopsis YUCCA1 flavin monooxygenase functions in the indole-3-pyruvic acid branch of auxin biosynthesis. Plant Cell. 2011 Nov;23(11):3961-73.

Stephan AB, Kunz HH, Yang E, Schroeder JI. Rapid hyperosmotic-induced Ca2+ responses in Arabidopsis thaliana exhibit sensory potentiation and involvement of plastidial KEA transporters. Proc Natl Acad Sci U S A. 2016 Aug 30;113(35):E5242-9.

Stirnberg P, van De Sande K, Leyser HM. MAX1 and MAX2 control shoot lateral branching in Arabidopsis. Development. 2002 Mar;129(5):1131-41.

Stirnberg P, Zhao S, Williamson L, Ward S, Leyser O. FHY3 promotes shoot branching and stress tolerance in Arabidopsis in an AXR1-dependent manner. Plant J. 2012 Sep;71(6):907-20.

Stock AM, Robinson VL, Goudreau PN. Two-component signal transduction. Annu Rev Biochem. 2000;69:183-215. Review.

Stone SL, Hauksdóttir H, Troy A, Herschleb J, Kraft E, Callis J. Functional analysis of the RING-type ubiquitin ligase family of Arabidopsis. Plant Physiol. 2005 Jan;137(1):13-30.

Su CH, Zhao R, Velazquez-Torres G, Chen J, Gully C, Yeung SC, Lee MH. Nuclear export regulation of COP1 by 14-3-3σ in response to DNA damage. Mol Cancer. 2010 Sep 15;9:243.

Su YH, Liu YB, Bai B, Zhang XS. Establishment of embryonic shoot-root axis is involved in auxin and cytokinin response during Arabidopsis somatic embryogenesis. Front Plant Sci. 2015 Jan 14;5:792.

Subramanian C, Kim BH, Lyssenko NN, Xu X, Johnson CH, von Arnim AG. The Arabidopsis repressor of light signaling, COP1, is regulated by nuclear exclusion: mutational analysis by bioluminescence resonance energy transfer. Proc Natl Acad Sci U S A. 2004 Apr 27;101(17):6798-802.

Sun TP, Gubler F. Molecular mechanism of gibberellin signaling in plants. Annu Rev Plant Biol. 2004;55:197-223. Review.

Sun Y, Fan XY, Cao DM, Tang W, He K, Zhu JY, He JX, Bai MY, Zhu S, Oh E, Patil S, Kim TW, Ji H, Wong WH, Rhee SY, Wang ZY. Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis. Dev Cell. 2010 Nov 16;19(5):765-77.

Sun Y, Li L, Macho AP, Han Z, Hu Z, Zipfel C, Zhou JM, Chai J. Structural basis for flg22-induced activation of the Arabidopsis FLS2-BAK1 immune complex. Science. 2013 Nov 1;342(6158):624-8.

Suzuki N. Hormone signaling pathways under stress combinations. Plant Signal Behav. 2016 Oct 14:0.

Suzuki T, Sakurai K, Ueguchi C, Mizuno T. Two types of putative nuclear factors that physically interact with histidine-containing phosphotransfer (Hpt) domains, signaling mediators in His-to-Asp phosphorelay, in Arabidopsis thaliana. Plant Cell Physiol. 2001 Jan;42(1):37-45.

Sweere U, Eichenberg K, Lohrmann J, Mira-Rodado V, Bäurle I, Kudla J, Nagy F, Schafer E, Harter K. Interaction of the response regulator ARR4 with phytochrome B in modulating red light signaling. Science. 2001 Nov 2;294(5544):1108-11.

Szekeres M, Németh K, Koncz-Kálmán Z, Mathur J, Kauschmann A, Altmann T, Rédei GP, Nagy F, Schell J, Koncz C. Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in Arabidopsis. Cell. 1996 Apr 19;85(2):171-82.

Taiz L, Zeiger E. Plant Physiology. 5th Edition. 2010; Sinauer Associates, Inc.

Takahashi K, Hayashi K, Kinoshita T. Auxin activates the plasma membrane H+-ATPase by phosphorylation during hypocotyl elongation in Arabidopsis. Plant Physiol. 2012 Jun;159(2):632-41.

Tan X, Calderon-Villalobos LI, Sharon M, Zheng C, Robinson CV, Estelle M, Zheng N. Mechanism of auxin perception by the TIR1 ubiquitin ligase. Nature. 2007 Apr 5;446(7136):640-5.

Tang W, Ji Q, Huang Y, Jiang Z, Bao M, Wang H, Lin R. FAR-RED ELONGATED HYPOCOTYL3 and FAR-RED IMPAIRED RESPONSE1 transcription factors integrate light and abscisic acid signaling in Arabidopsis. Plant Physiol. 2013 Oct;163(2):857-66.

Tang W, Kim TW, Oses-Prieto JA, Sun Y, Deng Z, Zhu S, Wang R, Burlingame AL, Wang ZY. BSKs mediate signal transduction from the receptor kinase BRI1 in Arabidopsis. Science. 2008 Jul 25;321(5888):557-60.

Tang W, Yuan M, Wang R, Yang Y, Wang C, Oses-Prieto JA, Kim TW, Zhou HW, Deng Z, Gampala SS, Gendron JM, Jonassen EM, Lillo C, DeLong A, Burlingame AL, Sun Y, Wang ZY. PP2A activates brassinosteroid-responsive gene expression and plant growth by dephosphorylating BZR1. Nat Cell Biol. 2011 Feb;13(2):124-31.

Tatematsu K, Kumagai S, Muto H, Sato A, Watahiki MK, Harper RM, Liscum E, Yamamoto KT. MASSUGU2 encodes Aux/IAA19, an auxin-regulated protein that functions together with the transcriptional activator NPH4/ARF7 to regulate differential growth responses of hypocotyl and formation of lateral roots in Arabidopsis thaliana. Plant Cell. 2004 Feb;16(2):379-93.

Teige M, Scheikl E, Eulgem T, Dóczi R, Ichimura K, Shinozaki K, Dangl JL, HirtH. The MKK2 pathway mediates cold and salt stress signaling in Arabidopsis. Mol Cell. 2004 Jul 2;15(1):141-52.

Tewson P, Westenberg M, Zhao Y, Campbell RE, Quinn AM, Hughes TE. Simultaneous detection of Ca2+ and diacylglycerol signaling in living cells. PLoS One. 2012;7(8):e42791.

Tezuka K, Taji T, Hayashi T, Sakata Y. A novel abi5 allele reveals the importance of the conserved Ala in the C3 domain for regulation of downstream genes and salt tolerance during germination in Arabidopsis. Plant Signal Behav. 2013 Mar;8(3):e23455.

Thimann KV. Hormones and the analysis of growth. Plant Physiol. 1938 Jul;13(3):437-49.

Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He SY, Howe GA, Browse J. JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. Nature. 2007 Aug 9;448(7154):661-5.

Thompson DS. Space and time in the plant cell wall: relationships between cell type, cell wall rheology and cell function. Ann Bot. 2008 Jan;101(2):203-11.

Tiwari SB, Hagen G, Guilfoyle T. The roles of auxin response factor domains in auxin-responsive transcription. Plant Cell. 2003 Feb;15(2):533-43.

Tiwari SB, Wang XJ, Hagen G, Guilfoyle TJ. AUX/IAA proteins are active repressors, and their stability and activity are modulated by auxin. Plant Cell. 2001 Dec;13(12):2809-22.

To JP, Haberer G, Ferreira FJ, Deruère J, Mason MG, Schaller GE, Alonso JM, Ecker JR, Kieber JJ. Type-A Arabidopsis response regulators are partially redundant negative regulators of cytokinin signaling. Plant Cell. 2004 Mar;16(3):658-71.

To JP, Kieber JJ. Cytokinin signaling: two-components and more. Trends Plant Sci. 2008 Feb;13(2):85-92. Review.

Toledo-Ortiz G, Johansson H, Lee KP, Bou-Torrent J, Stewart K, Steel G, Rodríguez-Concepción M, Halliday KJ. The HY5-PIF regulatory module coordinates light and temperature control of photosynthetic gene transcription. PLoS Genet. 2014 Jun 12;10(6):e1004416.

Törnroth-Horsefield S, Wang Y, Hedfalk K, Johanson U, Karlsson M, Tajkhorshid E, Neutze R, Kjellbom P. Structural mechanism of plant aquaporin gating. Nature. 2006 Feb 9;439(7077):688-94.

Torres-Franklin ML, Gigon A, de Melo DF, Zuily-Fodil Y, Pham-Thi AT. Drought stress and rehydration affect the balance between MGDG and DGDG synthesis in cowpea leaves. Physiol Plant. 2007 Oct;131(2):201-10.

Tosoni K, Costa A, Sarno S, D'Alessandro S, Sparla F, Pinna LA, Zottini M, Ruzzene M. The p23 co-chaperone protein is a novel substrate of CK2 in Arabidopsis. Mol Cell Biochem. 2011 Oct;356(1-2):245-54.

Tournaire-Roux C, Sutka M, Javot H, Gout E, Gerbeau P, Luu DT, Bligny R, Maurel C. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. Nature. 2003 Sep 25;425(6956):393-7.

Turk EM, Fujioka S, Seto H, Shimada Y, Takatsuto S, Yoshida S, Wang H, Torres QI, Ward JM, Murthy G, Zhang J, Walker JC, Neff MM. BAS1 and SOB7 act redundantly to modulate Arabidopsis photomorphogenesis via unique brassinosteroid inactivation mechanisms. Plant J. 2005 Apr;42(1):23-34.

Umezawa T, Sugiyama N, Mizoguchi M, Hayashi S, Myouga F, Yamaguchi-Shinozaki K, Ishihama Y, Hirayama T, Shinozaki K. Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in Arabidopsis. Proc Natl Acad Sci U S A. 2009 Oct 13;106(41):17588-93.

Urao T, Miyata S, Yamaguchi-Shinozaki K, Shinozaki K. Possible His to Asp phosphorelay signaling in an Arabidopsis two-component system. FEBS Lett. 2000 Aug 4;478(3):227-32.

Urao T, Yakubov B, Satoh R, Yamaguchi-Shinozaki K, Seki M, Hirayama T, Shinozaki K. A transmembrane hybrid-type histidine kinase in Arabidopsis functions as an osmosensor. Plant Cell. 1999 Sep;11(9):1743-54.

Veley KM, Maksaev G, Frick EM, January E, Kloepper SC, Haswell ES. Arabidopsis MSL10 has a regulated cell death signaling activity that is separable from its mechanosensitive ion channel activity. Plant Cell. 2014 Jul;26(7):3115-31.

Verica JA, He ZH. The cell wall-associated kinase (WAK) and WAK-like kinase gene family. Plant Physiol. 2002 Jun;129(2):455-9.

Verslues PE, Bray EA. Role of abscisic acid (ABA) and Arabidopsis thaliana ABA-insensitive loci in low water potential-induced ABA and proline accumulation. J Exp Bot. 2006;57(1):201-12.

Vetter MM, Kronholm I, He F, Häweker H, Reymond M, Bergelson J, Robatzek S, de Meaux J. Flagellin perception varies quantitatively in Arabidopsis thaliana and its relatives. Mol Biol Evol. 2012 Jun;29(6):1655-67.

Vlad F, Rubio S, Rodrigues A, Sirichandra C, Belin C, Robert N, Leung J, Rodriguez PL, Laurière C, Merlot S. Protein phosphatases 2C regulate the activation of the Snf1-related kinase OST1 by abscisic acid in Arabidopsis. Plant Cell. 2009 Oct;21(10):3170-84.

von Arnim AG, Deng XW. Light inactivation of Arabidopsis photomorphogenic repressor COP1 involves a cell-specific regulation of its nucleocytoplasmic partitioning. Cell. 1994 Dec 16;79(6):1035-45.

von Arnim AG, Osterlund MT, Kwok SF, Deng XW. Genetic and developmental control of nuclear accumulation of COP1, a repressor of photomorphogenesis in Arabidopsis. Plant Physiol. 1997 Jul;114(3):779-88.

Vu LD, Stes E, Van Bel M, Nelissen H, Maddelein D, Inzé D, Coppens F, Martens L, Gevaert K, De Smet I. Up-to-Date Workflow for Plant (Phospho)proteomics Identifies Differential Drought-Responsive Phosphorylation Events in Maize Leaves. J Proteome Res. 2016 Oct 4.

Wang C, Shang JX, Chen QX, Oses-Prieto JA, Bai MY, Yang Y, Yuan M, Zhang YL, Mu CC, Deng Z, Wei CQ, Burlingame AL, Wang ZY, Sun Y. Identification of BZR1-interacting proteins as potential components of the brassinosteroid signaling pathway in Arabidopsis through tandem affinity purification. Mol Cell Proteomics. 2013 Dec;12(12):3653-65.

Wang H, Wang H. Multifaceted roles of FHY3 and FAR1 in light signaling and beyond. Trends Plant Sci. 2015 Jul;20(7):453-61. Review.

Wang X, Chory J. Brassinosteroids regulate dissociation of BKI1, a negative regulator of BRI1 signaling, from the plasma membrane. Science. 2006 Aug 25;313(5790):1118-22.

Wang X, Kota U, He K, Blackburn K, Li J, Goshe MB, Huber SC, Clouse SD. Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. Dev Cell. 2008 Aug;15(2):220-35.

Wang X, Zhang J, Yuan M, Ehrhardt DW, Wang Z, Mao T. Arabidopsis microtubule destabilizing protein40 is involved in brassinosteroid regulation of hypocotyl elongation. Plant Cell. 2012a Oct;24(10):4012-25.

Wang Y, Li L, Ye T, Zhao S, Liu Z, Feng YQ, Wu Y. Cytokinin antagonizes ABA suppression to seed germination of Arabidopsis by downregulating ABI5 expression. Plant J. 2011a Oct;68(2):249-61.

Wang Y, Li Z, Liu D, Xu J, Wei X, Yan L, Yang C, Lou Z, Shui W. Assessment of BAK1 activity in different plant receptor-like kinase complexes by quantitative profiling of phosphorylation patterns. J Proteomics. 2014a Aug 28;108:484-93.

Wang Y, Shen W, Chan Z, Wu Y. Endogenous Cytokinin Overproduction Modulates ROS Homeostasis and Decreases Salt Stress Resistance in Arabidopsis Thaliana. Front Plant Sci. 2015a Nov 19;6:1004.

Wang Y, Shimazaki K, Kinoshita T. Multiple Roles of the Plasma Membrane H(+)-ATPase and Its Regulation. Enzymes. 2014b;35:191-211.

Wang Z, Meng P, Zhang X, Ren D, Yang S. BON1 interacts with the protein kinases BIR1 and BAK1 in modulation of temperature-dependent plant growth and cell death in Arabidopsis. Plant J. 2011b Sep;67(6):1081-93.

Wang ZY, Bai MY, Oh E, Zhu JY. Brassinosteroid signaling network and regulation of photomorphogenesis. Annu Rev Genet. 2012b;46:701-24. Review.

Wang ZY, Nakano T, Gendron J, He J, Chen M, Vafeados D, Yang Y, Fujioka S, Yoshida S, Asami T, Chory J. Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. Dev Cell. 2002 Apr;2(4):505-13.

Wang ZY, Seto H, Fujioka S, Yoshida S, Chory J. BRI1 is a critical component of a plasma-membrane receptor for plant steroids. Nature. 2001 Mar 15;410(6826):380-3. Erratum in: Nature 2001 May 10;411(6834):219.

Wasternack C, Hause B. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. Ann Bot. 2013 Jun;111(6):1021-58.

Wasternack C. Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Bot. 2007 Oct;100(4):681-97. Review.

Went FW, Thimann KV. Phytohormones. 1937. New York: MacMillan

West AH, Stock AM. Histidine kinases and response regulator proteins in two-component signaling systems. Trends Biochem Sci. 2001 Jun;26(6):369-76. Review.

Whitelam GC, Johnson E, Peng J, Carol P, Anderson ML, Cowl JS, Harberd NP. Phytochrome A null mutants of Arabidopsis display a wild-type phenotype in white light. Plant Cell. 1993 Jul;5(7):757-68.

Wilson ME, Maksaev G, Haswell ES. MscS-like mechanosensitive channels in plants and microbes. Biochemistry. 2013 Aug 27;52(34):5708-22. Review.

Wilson RS, Swatek KN, Thelen JJ. Regulation of the Regulators: Post-Translational Modifications, Subcellular, and Spatiotemporal Distribution of Plant 14-3-3 Proteins. Front Plant Sci. 2016 May 9;7:611.

Winter D, Vinegar B, Nahal H, Ammar R, Wilson GV, Provart NJ. An "Electronic Fluorescent Pictograph" browser for exploring and analyzing large-scale biological data sets. PLoS One. 2007 Aug 8;2(8):e718.

Wohlbach DJ, Quirino BF, Sussman MR. Analysis of the Arabidopsis histidine kinase ATHK1 reveals a connection between vegetative osmotic stress sensing and seed maturation. Plant Cell. 2008 Apr;20(4):1101-17

Won C, Shen X, Mashiguchi K, Zheng Z, Dai X, Cheng Y, Kasahara H, Kamiya Y, Chory J, Zhao Y. Conversion of tryptophan to indole-3-acetic acid by TRYPTOPHAN AMINOTRANSFERASES

OF ARABIDOPSIS and YUCCAs in Arabidopsis. Proc Natl Acad Sci U S A. 2011 Nov 8;108(45):18518-23.

Wood JM. Bacterial osmoregulation: a paradigm for the study of cellular homeostasis. Annu Rev Microbiol. 2011;65:215-38. Review.

Wu X, Sklodowski K, Encke B, Schulze WX. A kinase-phosphatase signaling module with BSK8 and BSL2 involved in regulation of sucrose-phosphate synthase. J Proteome Res. 2014 Jul 3;13(7):3397-409.

Wu XN, Sanchez Rodriguez C, Pertl-Obermeyer H, Obermeyer G, Schulze WX. Sucrose-induced receptor kinase SIRK1 regulates a plasma membrane aquaporin in Arabidopsis. Mol Cell Proteomics. 2013 Oct;12(10):2856-73.

Wu, XN, Schulze, WX. Phosphopeptide profiling of receptor kinase mutants. Meth.Mol. Biol. 2015 1306: 71-79.

Wulfetange K, Lomin SN, Romanov GA, Stolz A, Heyl A, Schmülling T. The cytokinin receptors of Arabidopsis are located mainly to the endoplasmic reticulum. Plant Physiol. 2011 Aug;156(4):1808-18.

Xiao B, Smerdon SJ, Jones DH, Dodson GG, Soneji Y, Aitken A, Gamblin SJ. Structure of a 14-3-3 protein and implications for coordination of multiple signalling pathways. Nature. 1995 Jul 13;376(6536):188-91.

Xie DX, Feys BF, James S, Nieto-Rostro M, Turner JG. COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. Science. 1998 May 15;280(5366):1091-4.

Xu D, Li J, Gangappa SN, Hettiarachchi C, Lin F, Andersson MX, Jiang Y, Deng XW, Holm M. Convergence of Light and ABA signaling on the ABI5 promoter. PLoS Genet. 2014a Feb 27;10(2):e1004197.

Xu DB, Chen M, Ma YN, Xu ZS, Li LC, Chen YF, Ma YZ. A G-protein β subunit, AGB1, negatively regulates the ABA response and drought tolerance by down-regulating AtMPK6-related pathway in Arabidopsis. PLoS One. 2015 Jan 30;10(1):e0116385.

Xu H, Liu Q, Yao T, Fu X. Shedding light on integrative GA signaling. Curr Opin Plant Biol. 2014b Oct;21:89-95. Review.

Xu X, Paik I, Zhu L, Bu Q, Huang X, Deng XW, Huq E. PHYTOCHROME INTERACTING FACTOR1 Enhances the E3 Ligase Activity of CONSTITUTIVE PHOTOMORPHOGENIC1 to Synergistically Repress Photomorphogenesis in Arabidopsis. Plant Cell. 2014c May 23;26(5):1992-2006.

Yamada H, Suzuki T, Terada K, Takei K, Ishikawa K, Miwa K, Yamashino T, Mizuno T. The Arabidopsis AHK4 histidine kinase is a cytokinin-binding receptor that transduces cytokinin signals across the membrane. Plant Cell Physiol. 2001 Sep;42(9):1017-23.

Yamauchi S, Takemiya A, Sakamoto T, Kurata T, Tsutsumi T, Kinoshita T, Shimazaki K. The Plasma Membrane H+-ATPase AHA1 Plays a Major Role in Stomatal Opening in Response to Blue Light. Plant Physiol. 2016 Aug;171(4):2731-43.

Yan Y, Stolz S, Chételat A, Reymond P, Pagni M, Dubugnon L, Farmer EE. A downstream mediator in the growth repression limb of the jasmonate pathway. Plant Cell. 2007 Aug;19(8):2470-83.

Yaneff A, Sigaut L, Gómez N, Aliaga Fandiño C, Alleva K, Pietrasanta LI, Amodeo G. Loop B serine of a plasma membrane aquaporin type PIP2 but not PIP1 plays a key role in pH sensing. Biochim Biophys Acta. 2016 Aug 10;1858(11):2778-2787..

Yang Y, Hammes UZ, Taylor CG, Schachtman DP, Nielsen E. High-affinity auxin transport by the AUX1 influx carrier protein. Curr Biol. 2006 Jun 6;16(11):1123-7.

Yanovsky MJ, Kay SA. Living by the calendar: how plants know when to flower. Nat Rev Mol Cell Biol. 2003 Apr;4(4):265-75. Review.

Yanovsky MJ, Whitelam GC, Casal JJ. fhy3-1 retains inductive responses of phytochrome A. Plant Physiol. 2000 May;123(1):235-42.

Yeh KC, Lagarias JC. Eukaryotic phytochromes: light-regulated serine/threonine protein kinases with histidine kinase ancestry. Proc Natl Acad Sci U S A. 1998 Nov 10;95(23):13976-81.

Yennawar NH, Li LC, Dudzinski DM, Tabuchi A, Cosgrove DJ. Crystal structure and activities of EXPB1 (Zea m 1), a beta-expansin and group-1 pollen allergen from maize. Proc Natl Acad Sci U S A. 2006 Oct 3;103(40):14664-71.

Yin Y, Wang ZY, Mora-Garcia S, Li J, Yoshida S, Asami T, Chory J. BES1 accumulates in the nucleus in response to brassinosteroids to regulate gene expression and promote stem elongation. Cell. 2002 Apr 19;109(2):181-91.

Yoo SD, Cho YH, Sheen J. Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. Nat Protoc. 2007;2(7):1565-72.

Yoshida T, Mogami J, Yamaguchi-Shinozaki K. ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. Curr Opin Plant Biol. 2014 Oct;21:133-9. Review.

Yu F, Li J, Huang Y, Liu L, Li D, Chen L, Luan S. FERONIA receptor kinase controls seed size in Arabidopsis thaliana. Mol Plant. 2014 May;7(5):920-2.

Yu H, Zhang Y, Moss BL, Bargmann BO, Wang R, Prigge M, Nemhauser JL, Estelle M. Untethering the TIR1 auxin receptor from the SCF complex increases its stability and inhibits auxin response. Nat Plants. 2015 Mar;1(3).

Yuan TT, Xu HH, Zhang KX, Guo TT, Lu YT. Glucose inhibits root meristem growth via ABA INSENSITIVE 5, which represses PIN1 accumulation and auxin activity in Arabidopsis. Plant Cell Environ. 2014 Jun;37(6):1338-50.

Zauber H, Schulze WX. Proteomics wants cRacker: automated standardized data analysis of LC-MS derived proteomic data. J Proteome Res. 2012 Nov 2;11(11):5548-55.

Zhao Y, Araki S, Wu J, Teramoto T, Chang YF, Nakano M, Abdelfattah AS, Fujiwara M, Ishihara T, Nagai T, Campbell RE. An expanded palette of genetically encoded $Ca^{2+}$ indicators. Science. 2011 Sep 30;333(6051):1888-91.

Zheng L, Liu G, Meng X, Liu Y, Ji X, Li Y, Nie X, Wang Y. A WRKY gene from Tamarix hispida, ThWRKY4, mediates abiotic stress responses by modulating reactive oxygen species and expression of stress-responsive genes. Plant Mol Biol. 2013a Jul;82(4-5):303-20.

Zheng X, Wu S, Zhai H, Zhou P, Song M, Su L, Xi Y, Li Z, Cai Y, Meng F, Yang L, Wang H, Yang J. Arabidopsis phytochrome B promotes SPA1 nuclear accumulation to repress photomorphogenesis under far-red light. Plant Cell. 2013b Jan;25(1):115-33.

Zhou X, Hao H, Zhang Y, Bai Y, Zhu W, Qin Y, Yuan F, Zhao F, Wang M, Hu J, Xu H, Guo A, Zhao H, Zhao Y, Cao C, Yang Y, Schumaker KS, Guo Y, Xie CG. SOS2-LIKE PROTEIN KINASE5, an SNF1-RELATED PROTEIN KINASE3-Type Protein Kinase, Is Important for Abscisic Acid Responses in Arabidopsis through Phosphorylation of ABSCISIC ACID-INSENSITIVE5. Plant Physiol. 2015 Jun;168(2):659-76.

Zhou XE, Soon FF, Ng LM, Kovach A, Suino-Powell KM, Li J, Yong EL, Zhu JK, Xu HE, Melcher K. Catalytic mechanism and kinase interactions of ABA-signaling PP2C phosphatases. Plant Signal Behav. 2012 May;7(5):581-8.

Zhou XY, Song L, Xue HW. Brassinosteroids regulate the differential growth of Arabidopsis hypocotyls through auxin signaling components IAA19 and ARF7. Mol Plant. 2013 May;6(3):887-904.

Zhou Y, Yang Z, Guo G, Guo Y. Microfilament dynamics is required for root growth under alkaline stress in Arabidopsis. J Integr Plant Biol. 2010 Nov;52(11):952-8.

Zipfel C, Robatzek S, Navarro L, Oakeley EJ, Jones JD, Felix G, Boller T. Bacterial disease resistance in Arabidopsis through flagellin perception. Nature. 2004 Apr 15;428(6984):764-7.

Zuo J, Niu QW, Chua NH. Technical advance: An estrogen receptor-based transactivator XVE mediates highly inducible gene expression in transgenic plants. Plant J. 2000 Oct;24(2):265-73.

# 7 CURRICULUM VITAE

## Personal Information

| | |
|---|---|
| name | Rebecca Dautel |
| date of birth | 25th October 1986 |
| place of birth | Filderstadt, Germany |
| nationality | German |
| personal status | single |

## Education

since 04/2012 — **Ph.D. in Molecular Biology**
University of Tübingen, Germany
Center for Plant Molecular Biology (ZMBP)
Department of Plant Physiology

09/2006 - 02/2012 — **Diploma Studies** and **Diploma Degree in Biology**
University of Tübingen, Germany
Title: Molekulare Charakterisierung synthetischer Promotoren am Beispiel des $P_{syn}$ und des putativ bidirektionalen *Raf42/Raf43*-Promotors aus *Arabidopsis thaliana*

09/1997 - 07/2006 — **Secondary school**
Eduard-Spranger-Gymnasium, Filderstadt, Germany

09/1993 - 07/1997 — **Primary school**
Bruckenacker Grundschule, Filderstadt, Germany

## Visting Research Fellowships

05/2012 — **Max Planck Institute for Molecular Plant Physiology**, Golm, Germany
Cooperation with Prof. Dr. Waltraud X. Schulze
Research fellowship to familiarize with the method of phosphoenrichment and the analysis of LC-MS/MS-data.

06/2013 — **University of Nottingham**, England
Cooperation with Prof. Dr. Malcolm J. Bennett
Research fellowship to learn and convey hydrotropism, gravitropism and thigmotropism assays as well as the method to analyze lateral root development.

04/2015 — **University of Salzburg**, Austria
Cooperation with Prof. Dr. Gerhard Obermeyer
Research fellowship to learn and convey protoplast swelling assays.

## Publications and Manuscripts

Hahn A, Kilian J, Mohrholz A, Ladwig F, Peschke F, Dautel R, Harter K, Berendzen KW, Wanke D. Plant Core Environmental Stress Response Genes Are Systematically Coordinated during Abiotic Stresses. Int J Mol Sci. 2013 Apr 8;14(4):7617-41.

Dautel R, Wu XN, Heunemann M, Schulze WX, Harter K. The Sensor Histidine Kinases AHK2 and AHK3 Proceed into Multiple Serine/Threonine/Tyrosine Phosphorylation Pathways in *Arabidopsis thaliana*. Mol Plant. 2016 Jan 4;9(1):182-6.

Dautel R, Schulze WX, Obermeyer G, Harter K. AHK1 contributes to osmoregulation by mediating changes in multiple Serine/Threonine/Tyrosine Phosphorylation Pathways in *Arabidopsis thaliana*. (in preparation).

## Talks

Dautel R (2012): Use of a fully synthetic promoter as tool for hormone-signaing research. 1st Summer Academy in plant molecular biology, Freudenstadt

## Poster Presentations

Dautel R; Wanke D; Mohrholz A (2011): A non-natural promoter as model for gene expression studies. Botanikertagung, Berlin

Dautel R; Wanke D; Mohrholz A (2011): A non-natural promoter as model for gene expression studies. 5th Regio Plant Science Meeting Stuttgart - Tübingen - Ulm

Dautel R, Hothorn M, Caesar C, Wanke D, Harter K (2014): Evolutionary relationships, putative structure, intracellular localization and cellular function of AHK1. 27. Tagung Molekularbiologie der Pflanzen, Dabringhausen

Dautel R, Hothorn M, Caesar C, Wanke D, Harter K (2014): Evolutionary relationships, putative structure, intracellular localization and cellular function of AHK1. International Meeting of the German Society for Cell Biology (DGZ), Regensburg

Dautel R, Caesar K, Schulze WX, Hothorn M, Harter K (2016): Localization, intracellular dynamics and cellular function of the *Arabidopsis thaliana* histidine kinase AHK1. 29. Tagung Molekularbiologie der Pflanzen, Dabringhausen
**This poster was awarded with the Reinhold von Sengbusch Poster Award.**

# 8 APPENDIX

## A1: Vectors which have been provided for the Ph.D. thesis

Entry vectors and vectors for further cloning

| vector name (source) | selection | purpose |
|---|---|---|
| *pENTR/D-Topo* (invitrogen) | Kan | Gateway™-cloning |
| *pDONR207* (invitrogen) | Gent | Gateway™-cloning |
| *pENTR/D-Topo-AHK3* (Jakub Horak) | Kan | Gateway™-cloning |
| *pDONR207-AHK4* (Julia Teply) | Gent | Gateway™-cloning |

*E.coli* expression vectors

| vector name (source) | selection | purpose |
|---|---|---|
| *pMH-HSsumo_AHK4* (Michael Hothorn) | Kan | *E. coli* expression vector |

Vectors for protein interaction studies with *Saccharomyces cerevisiae*

| vector name (source) | selection (*E coli* / yeast) | purpose |
|---|---|---|
| *pMetYC-Dest* (Christopher Grefen) | Amp, Cm / Leu | Cub-fusion, Gateway™-cloning |
| *pMetYC-AHK1* (Christopher Grefen) | Amp / Leu | mbSUS, AHK1-Cub |
| *pMetYC-AHK2* (Christopher Grefen) | Amp / Leu | mbSUS, AHK2-Cub |
| *pMetYC-AHK4* (Christopher Grefen) | Amp / Leu | mbSUS, AHK4-Cub |
| *pMetYC-CKI1* (Christopher Grefen) | Amp / Leu | mbSUS, CKI1-Cub |
| *pMetYC-BAK1* (Peter Huppenberger) | Amp / Leu | mbSUS, BAK1-Cub |
| *pMetYC-BRI1* (Christopher Grefen) | Amp / Leu | mbSUS,BRI1-Cub |
| *pMetYC-AHA1* (Friederike Wanke) | Amp / Leu | mbSUS, AHA1-Cub |
| *pXNubA22-Dest* (Christopher Grefen) | Amp, Cm / Trp | Nub-fusion, Gateway™-cloning |
| *pXNubA22-AHK1* (Christopher Grefen) | Amp / Trp | mbSUS, AHK1-Nub |
| *pXNubA22-ETR1* (Christopher Grefen) | Amp / Trp | mbSUS,ETR1-Nub |
| *pXNubA22-AHK5_fl* (Michael Heunemann) | Amp / Trp | mbSUS, AHK1_fl-Nub |
| *pXNubA22-AHK5_C8* (Michael Heunemann) | Amp / Trp | mbSUS, AHK1_C8-Nub |
| *pXNubA22-CKI1* (Christopher Grefen) | Amp / Trp | mbSUS, CKI1-Nub |
| *pXNubA22-BAK1* (Peter Huppenberger) | Amp / Trp | mbSUS, BAK1-Nub |
| *pXNubA22-BRI1* (Peter Huppenberger) | Amp / Trp | mbSUS, BRI1-Nub |
| *pXNubA22-AHA1* (Friederike Wanke) | Amp / Trp | mbSUS, AHA1-Nub |
| *pXNubA22-AHA2* (Friederike Wanke) | Amp / Trp | mbSUS, AHA2-Nub |
| *pXNubA22-RLP44* (Friederike Wanke) | Amp / Trp | mbSUS, RLP44-Nub |
| *pXNubA22-CNGC17* (Friederike Wanke) | Amp / Trp | mbSUS, CNGC17-Nub |
| *pX32-Dest* (Christopher Grefen) | Amp, Cm / Trp | mbSUS, NubG |
| *pNubWtXgate* (Christopher Grefen) | Amp, Cm / Trp | mbSUS, NubWT |
| *pGBKT7-Dest* (Achim Hahn) | Kan, Cm / Trp | BD-fusion, Gateway™-cloning |
| *pGADT7-Dest* (Achim Hahn) | Amp, Cm / Leu | AD-fusion, Gateway™-cloning |
| *pGADT7-MPK2* (Achim Hahn) | Amp / Leu | Y2H, AD-MPK2 |
| *pGADT7-MPK3* (Achim Hahn) | Amp / Leu | Y2H, AD-MPK3 |

| vector name (source) | selection (*E coli* / yeast) | purpose |
|---|---|---|
| *pGADT7-MPK4* (Achim Hahn) | Amp / Leu | Y2H, AD-MPK4 |
| *pGADT7-MPK5* (Achim Hahn) | Amp / Leu | Y2H, AD-MPK5 |
| *pGADT7-MPK6* (Achim Hahn) | Amp / Leu | Y2H, AD-MPK6 |
| *pGADT7-MPK7* (Achim Hahn) | Amp / Leu | Y2H, AD-MPK7 |
| *pGADT7-MPK11* (Achim Hahn) | Amp / Leu | Y2H, AD-MPK11 |
| *pGADT7-MPK17* (Achim Hahn) | Amp / Leu | Y2H, AD-MPK17 |

Binary plant vectors

| vector name (source) | selection *E. coli* / plants) | purpose |
|---|---|---|
| *pB7-AHK1pro-mCherryNLS* (Katharina Caesar) | Spec / Basta | test AHK1 promoter |
| *pH7FWG2-AHK1* (Jakub Horak) | Spec / Hyg | *35S::AHK1-GFP* |
| *pABind-AHK1-GFP* | Spec / Hyg | *lexA$^{-46}$35S::AHK1-GFP* |
| *pB7RWG2-CKI1* | Spec / Basta | *35S::CKI1-RFP* |
| *pBT8-ARR5::LUCm3* (Niklas Wallmeroth) | Amp | *ARR5::LUC5* |
| *pB7-RD29Bpro-LUCm-3XHA* (Manikandan Veerabagu) | Spec / Basta | *RD29B::LUC* |
| *pFRK::LUC nos c* (Markus Albert) | Amp | *pFRK1::LUC* |
| *pUBN-RFP-MBD (Sabine Müller)* | Spec / Basta | *pUBQ::RFP-MBD* |
| *pUB-GFP-ABD2-GFP (Sabine Müller)* | Spec / Basta | *pUBQ::GFP-ABD2-GFP* |
| *pGPTVII-Bar-U-RGECO1* (Karin Schuhmacher) | Kan / Basta | *pUBQ10::R-GECO1* |
| | | |
| *CD3-959 er-rk* (Andreas Nebenführ) | Kan / Kan | marker ER |
| *CD3-967 g-rk* (Andreas Nebenführ) | Kan / Kan | marker golgi |
| *CD3-975 vac-rk* (Andreas Nebenführ) | Kan / Kan | marker vacuole |
| *CD3-983 px-rk* (Andreas Nebenführ) | Kan / Kan | marker peroxisomes |
| *CD3-991 mt-rk* (Andreas Nebenführ) | Kan / Kan | marker mitochondria |
| *CD3-999 pt-rk* (Andreas Nebenführ) | Kan / Kan | marker plastids |
| *CD3-1007 pm-rk* (Andreas Nebenführ) | Kan / Kan | marker plasma membrane |
| | | |
| *pABind-ARA6-mCherry* (Stephanie Hahn, Peter Pimpl) | Spec / Hyg | marker late endosome, prevacuolar compartment |
| *pABind-ARA7-mCherry* (Stephanie Hahn, Peter Pimpl) | Spec / Hyg | marker late endosome, prevacuolar compartment |
| *pABind-PEP12-mCherry* (Stephanie Hahn, Peter Pimpl) | Spec / Hyg | marker post-golgi compartment |
| *pABind-RabA5d-mCherry* (Stephanie Hahn,Peter Pimpl) | Spec / Hyg | marker recycling endosome |
| *pABind-RabA1e-mCherry* (Stephanie Hahn, Peter Pimpl) | Spec / Hyg | marker recycling endosome |
| *pABind-RabD2a-mCherry* (Stephanie Hahn, Peter Pimpl) | Spec / Hyg | marker endosome, golgi-system |
| *pABind-VTI12-mCherry* (Stephanie Hahn, Peter Pimpl) | Spec / Hyg | marker TGN, early endosome |
| *pABind-GOT1-mCherry* (Stephanie Hahn, Peter Pimpl) | Spec / Hyg | marker golgi |

## A2: Vectors which have been generated during the Ph.D. thesis

Entry vectors and vectors for further cloning

| vector name | selection | purpose |
|---|---|---|
| *pUC57-AHK1-ED* (GenScript) | Amp | codon-optimized (c.o.) AHK1-ED |
| *pUC57-AHK1-ED-Leu298Ala* | Amp | c.o. AHK1-ED with Leu298 mutated to Ala |
| *pUC57-AHK1-ED-Leu422Ala* | Amp | c.o. AHK1-ED with Leu422 mutated to Ala |
| *pUC57-AHK1-ED-Leu298/422Ala* | Amp | c.o. AHK1-ED with Leu298 and Leu422 mutated to Ala |
| *pENTR/D-Topo-MKKK20*+stop | Kan | Gateway™-cloning |
| *pENTR/D-Topo-MKKK20-stop* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-AT1G80350+stop* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-AT1G80350-stop* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-AT1G72250+stop* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-AT1G72250-stop* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-AT1G14390-stop* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-AT1G04780+stop* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-AT5G20470+stop* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-AHK1-ICP* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-AHK1-ED* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-IAA16+stop* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-IAA16-stop* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-IAA16-stop-S150A* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-IAA16-stop-S150E* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-WAK4+stop* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-WAK4-stop* | Kan | Gateway™-cloning |
| *pENTR/D-Topo-BIR1* | Kan | Gateway™-cloning |
| *pDONR207-BRI1* | Gent | Gateway™-cloning |
| *pDONR207-BRI1-S1172A* | Gent | Gateway™-cloning |
| *pDONR207-BRI1-S1172E* | Gent | Gateway™-cloning |

*E.coli* expression vectors

| vector name (producer) | selection | purpose |
|---|---|---|
| *pMH-HSsumo-AHK1-ED* | Amp | *E. coli* expression vector for AHK1-ED |
| *pMH-HSsumo-AHK1-ED-Leu298/422Ala* | Amp | *E. coli* expression vector for mutated AHK1-ED |

Vectors for protein interaction studies with *Saccharomyces cerevisiae*

| vector name (producer) | selection (*E. coli* / yeast) | purpose |
|---|---|---|
| *pMetYC-MKKK20* | Amp / Leu | mbSUS, MKKK20-Cub |
| *pMetYC-AT1G80350* | Amp / Leu | mbSUS, AT1G80350-Cub |
| *pMetYC-AT1G72250* | Amp / Leu | mbSUS, AT1G72250-Cub |
| *pMetYC-AT1G14390* | Amp / Leu | mbSUS, AT1G14390-Cub |
| *pMetYC-IAA16* | Amp / Leu | mbSUS, IAA16-Cub |
| *pMetYC-BIR1* | Amp / Leu | mbSUS, BIR1-Cub |
| *pMetYC-WAK4* | Amp / Leu | mbSUS, WAK4-Cub |
| *pXNubA22-AHK3* | Amp / Trp | mbSUS, AHK3-Nub |

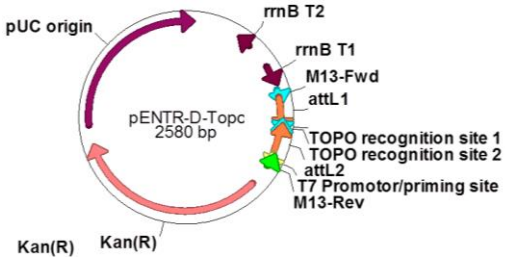
| vector name (producer) | selection (*E. coli* / yeast) | purpose |
|---|---|---|
| *pXNubA22-AHK4* | Amp / Trp | mbSUS, AHK4-Nub |
| *pXNubA22-MKKK20* | Amp / Trp | mbSUS, MKKK20-Nub |
| *pXNubA22-AT1G80350* | Amp / Trp | mbSUS, AT1G80350-Nub |
| *pXNubA22-AT1G72250* | Amp / Trp | mbSUS, AT1G72250-Nub |
| *pXNubA22-AT1G14390* | Amp / Trp | mbSUS, AT1G14390-Nub |
| *pXNubA22-IAA16* | Amp / Trp | mbSUS, IAA16-Nub |
| *pXNubA22-BIR1* | Amp / Trp | mbSUS, BIR1-Nub |
| *pXNubA22-WAK4* | Amp / Trp | mbSUS, WAK4-Nub |
| | | |
| *pGBKT7-AHK1-ICP* | Kan / Trp | Y2H, BD-AHK1-ICP |
| *pGBKT7-AHK1-ED* | Kan / Trp | Y2H, BD-AHK1-ED |
| *pGBKT7-IAA16* | Kan / Trp | Y2H, BD-IAA16 |
| *pGBKT7-WAK4* | Kan / Trp | Y2H, BD-WAK4 |
| | | |
| *pGADT7-AHK1-ICP* | Amp / Leu | Y2H, AD-AHK1-ICP |
| *pGADT7-AHK1-ED* | Amp / Leu | Y2H, AD-AHK1-ED |
| *pGADT7-AT1G04780* | Amp / Leu | Y2H, AD-AT1G04780 |
| *pGADT7-AT1G80350* | Amp / Leu | Y2H, AD-AT1G80350 |
| *pGADT7-AT1G72250* | Amp / Leu | Y2H, AD-AT1G72250 |
| *pGADT7-AT5G20470* | Amp / Leu | Y2H, AD-AT5G20470 |
| *pGADT7/-IAA16* | Amp / Leu | Y2H, AD-IAA16 |
| *pGADT7-WAK4* | Amp / Leu | Y2H, AD-WAK4 |
| *pGADT7-MKKK20* | Amp / Leu | Y2H, AD-MKKK20 |

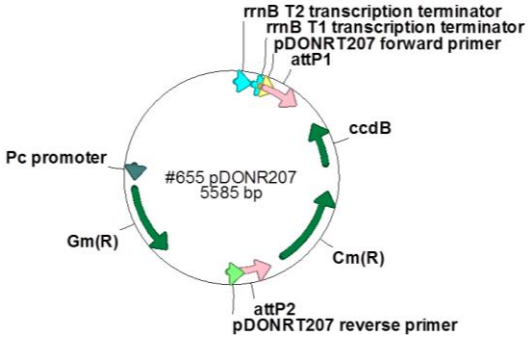
Binary plant vectors

| vector name (producer) | selection (*E. coli* / plants) | purpose |
|---|---|---|
| *pB7WGR2-IAA16* | Spec / Basta | *35S::RFP-IAA16* |
| *pH7WGF2-IAA16* | Spec / Hyg | *35S::GFP-IAA16* |
| pB7WGR2-IAA16-S150A | Spec / Basta | *35S::RFP-IAA16-S150A* |
| pB7WGR2-IAA16-S150E | Spec / Basta | *35S::RFP-IAA16-S150E* |
| pB7RWG2-BRI1 | Spec / Basta | *35S::BRI-RFP* |
| pH7FWG2-BRI1 | Spec / Hyg | *35S::BRI1-GFP* |
| pB7RWG2-BIR1 | Spec / Basta | *35S::BIR1-RFP* |
| pH7FWG2-BIR1 | Spec / Hyg | *35S::BIR1-GFP* |

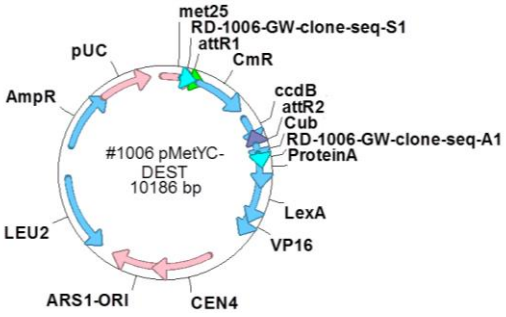
## A3: Vector maps

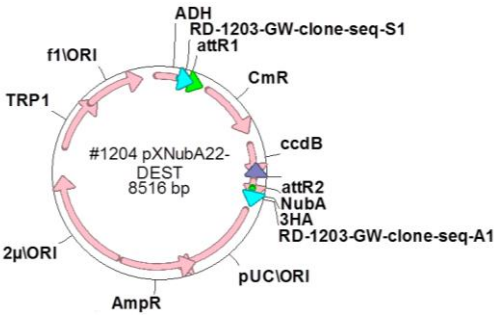
Entry-vectors



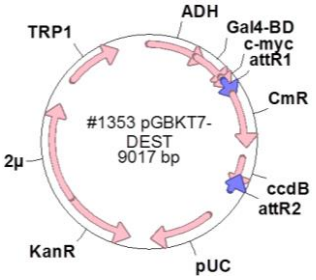



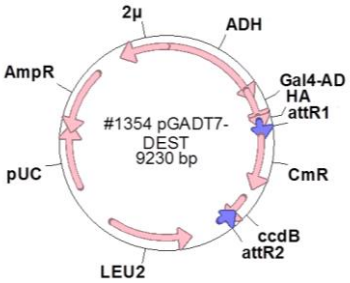
Destination-vectors for mating-based split-ubiquitin assays



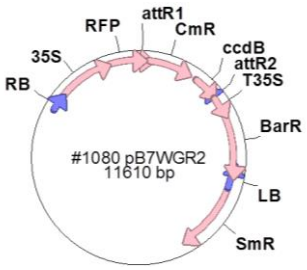



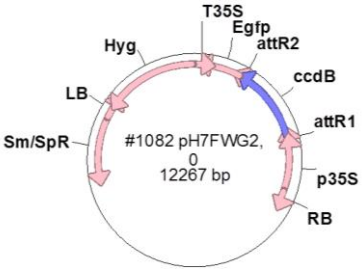

Destination vectors for yeast two-hybrid assays

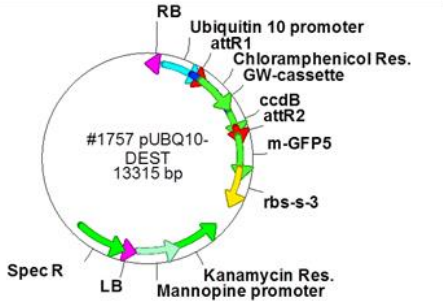


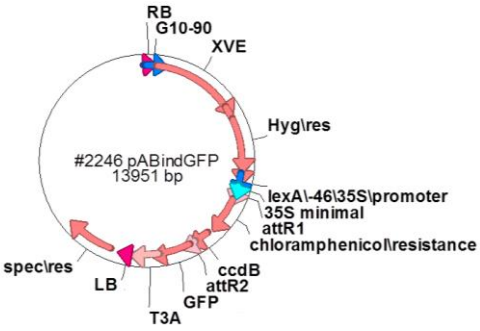




Binary plant destination-vectors

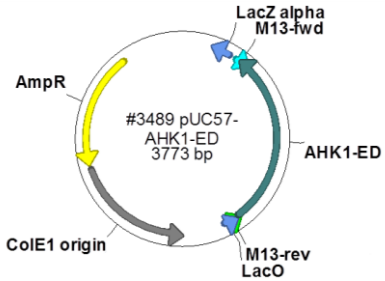


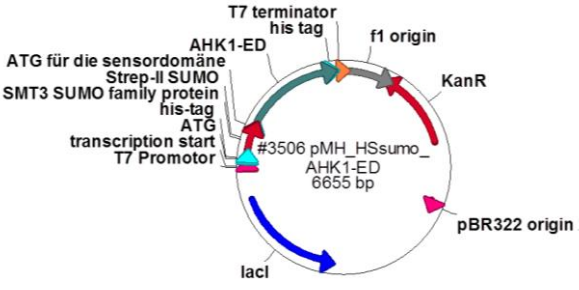



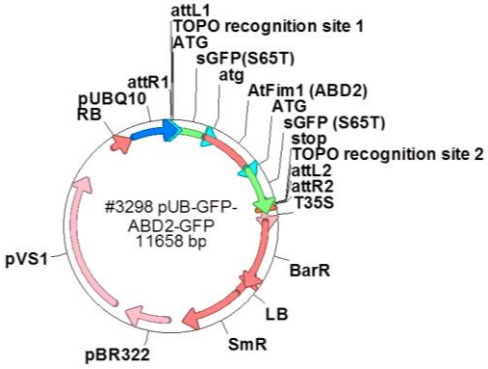



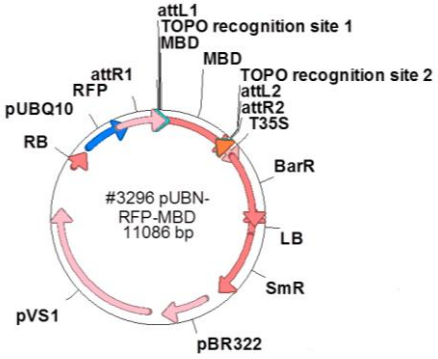

*pUC57* with codon-optimized *AHK1-ED* and vector with codon-optimized *AHK1-ED* for expression of AHK1-ED in *Escherichia coli*

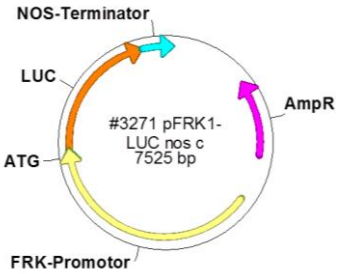


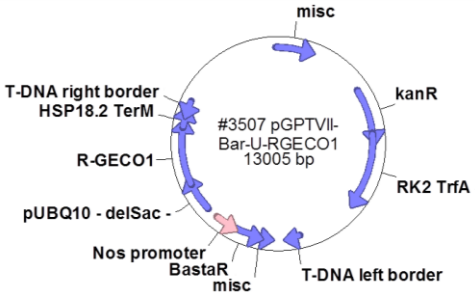



Vectors which were provided by partners of cooperations




## A4: Oligonucleotides for genotyping of *Arabidopsis thaliana* T-DNA insertion lines

| genotype | purpose | DNA-sequence |
|---|---|---|
| *ahk1-1* | T-DNA | TCCGTTCCGTTTTCGTTTTTTAC |
| | gene-specific Fwd | TCTGGTATATTCTGTGATTACTCTACAG |
| | gene-specific Rev | AAACTTTAGTAGACAAATCAGAAACCCA |
| *ahk1-3* | T-DNA | CATTTTATAATAACGCTGCGGACATCTAC |
| | gene-specific Fwd | GACCTCTCTGGTATGACTCGGTATTATA |
| | gene-specific Rev | CACATCCAGTATCATCAACCTCAAACCA |
| *ahk1-4* | T-DNA | CATTTTATAATAACGCTGCGGACATCTAC |
| | gene-specific Fwd | AGGAAGGTGTTCGATAAAATGACTGAATG |
| | gene-specific Rev | CAAGTTCTTCTTGAGTTGTTGGCTTGTCA |
| *ahk1-5* | T-DNA | AACGTCCGCAATGTGTTATTAAGTTGTC |
| | gene-specific Fwd | TATTATTACAAACATATTCCTCTCTATA |
| | gene-specific Rev | GATCCCAAATCATAAACAAAGACACATA |
| *ahk1-6* | T-DNA | AACGTCCGCAATGTGTTATTAAGTTGTC |
| | gene-specific Fwd | TCTGGTATATTCTGTGATTACTCTACAG |
| | gene-specific Rev | GTTAAAAGCCCTATCAAAATTGCTAACA |
| *bak1-1* | T-DNA | CATTTTATAATAACGCTGCGGACATCTAC |
| | gene-specific Fwd | CTATTTGGCGACACTACTTTCTGAC |
| | gene-specific Rev | GGTGCTTCAAAGTTGGGATGC |
| *bak1-3* | T-DNA | TGGTTCACGTAGTGGGCCATCG |
| | gene-specific Fwd | CTATTTGGCGACACTACTTTCTGAC |
| | gene-specific Rev | GGTGCTTCAAAGTTGGGATGC |
| *bak1-4* | T-DNA | TGGTTCACGTAGTGGGCCATCG |
| | gene-specific Fwd | ACATCATCATCATTCGCGAGG |
| | gene-specific Rev | TTATTGTTTGGCCGATCTTGG |

| genotype | purpose | DNA-sequence |
|---|---|---|
| *cngc7* | T-DNA | GCGTGGACCGCTTGCTGCAACT |
| | gene-specific Fwd | CCTTATTATGGCCCATCAACATAAGA |
| | gene-specific Rev | GGATCGGCAACAAATACTTAAAGTGA |
| *aha1-6* | T-DNA | TCAAACAGGATTTTCGCCTGC |
| | gene-specific Fwd | CGTCTCAACAAAAGTCTCTTTC |
| | gene-specific Rev | CGAAAGATCAACCTCGTGAG |
| *aha2-4* | T-DNA | TCAAACAGGATTTTCGCCTGC |
| | gene-specific Fwd | ATGTTCATTGCAAAGGTGGT |
| | gene-specific Rev | CCCATTAGCTCGTGGTTATT |
| *ahk2-2* | T-DNA | ATAACGCTGCGGACATCTAC |
| | gene-specific Fwd | GTCTATAACTTGTGAGCTCTTGAATC |
| | gene-specific Rev | GCTCGTGTCATAGACAGCAAAGGTC |
| *ahk3-3* | T-DNA | TGGTTCACGTAGTGGGCCATCG |
| | gene-specific Fwd | CTTGTGATTGCGTTACTTGTTGCAC |
| | gene-specific Rev | GCAGGCCTATGGTCCACAACCACAG |

## A5: Oligonucleotides and restriction endonucleases (REs) for genotyping of *Arabidopsis thaliana* EMS mutants

| genotype | DNA-sequence (Fwd / Rev) | RE |
|---|---|---|
| *bri1-5* | TTTCATTTCAAGCTTCACCATCTCAG / AGAGATGTTCAACAACTTGAGCTCTG | HpyCH4V |
| *bri1-301* | ATGGAAACCATTGGGAAGATCAAACA / CTTCATAAGCTCGGGGTCAAACACA | Bsp143I |

## A6: Oligonucleotides for the detection of T-DNAs in stably transformed *Arabidopsis thaliana* lines

| insert | DNA-sequence (Fwd / Rev) |
|---|---|
| *35S::AHK1-GFP* | TATGGAAGTACAGCAAGAATGAT / TTACTTGTACAGCTCGTCCATGC |
| *UBQ10::AHK1-GFP* | TATGGAAGTACAGCAAGAATGAT / TTTGTATAGTTCATCCATGCCATGTG |
| *RFP-MBD* | ATCTCCCTCAACTCTTGTTTCCAC / GTCCTTGGAAACACACTTGGATTG |
| *GFP-ABD2-GFP* | GAAAGGAATGGTCTAAACAAGGATGG / GAGGAAGAATAACCCACTCGATAGAC |
| *R-GECO1* | GTCGACATCAAGTTGGACATCGTG / CCTTGTAGATGAATACGCCGTCCT |

## A7: Oligonucleotides for cloning

| purpose | DNA-sequence (Fwd / Rev) |
|---|---|
| MKKK20-stop | CACCAGCTTTTGTTTCAATTTCAATGGAG/CCCTAGCCTTCCAAACACACTG |
| AT1G80350-stop | CACCATGGTGGGAAGTAGTAATTCG/AGCAGATCCAAACTCAGAGAGCCAC |
| AT1G80350+stop | CACCATGGTGGGAAGTAGTAATTCG/TAATTAAGCAGATCCAAACTCAGAGAGCCAC |
| AT1G72250-stop | CACCTTGTTAATGGAGGATTGTTGTGA/GATCCATCGCTCTTGTTTCTGCGG |
| AT1G72250+stop | CACCTTGTTAATGGAGGATTGTTGTGA/CAGTTTTTAATAGTTTCAGATCCATCGCTC |
| AT1G14390-stop | CACCATGCATAGTTCCTCTAAAAGCCAGG/TAGTTCTGAACCACCAAGCCCGAGG |
| AT1G04780+stop | CACCATGGCAAGCAGCACCATTGATG/CTTTTTCTTCAGCTGTTCTGAGATGAC |
| AT5G20470+stop | CACCATGCATCAGGACATGGTTCTAC/TTATGATGGACTAGCTTCTTTACGAGTGAG |
| AHK1-ICP+stop | CACCAATGGAACCGGTGTTTCAAAGGAGA/GGTCAAGCGGACAATGAAGTTTGGAA |
| AHK1-ED+stop | CACCTGGCATTTCACAAGGATTTATACAAAGCAG/<br>CTAAGTCTTGAAGGCCCTTTCATCCACTTTTCCCA |

| purpose | DNA-sequence (Fwd / Rev) |
|---|---|
| IAA16+stop | CACCATGATTAATTTTGAGGCCACGGAGC/TCAACTTCTGTTCTTGCACTTTTCTAATG |
| ARF21+stop | CACCATGGAAAGTGGCAACATTGTGAA/TCAACTTGAGAGACTCTTACTGGAC |
| WAK4+stop | CACCATGAAAGTGCAGCGTCTGTTCTTAG/TCAGCGGCCTGCTTCAATGTCCAG |
| BIR1-stop | CACCATGATGATGGGTAGGTTAGTTTTTGTAA/<br>ACGAGCAACTATGAGCTCTTCAATGAAA |

## A8: Oligonucleotides for site-directed mutagenesis

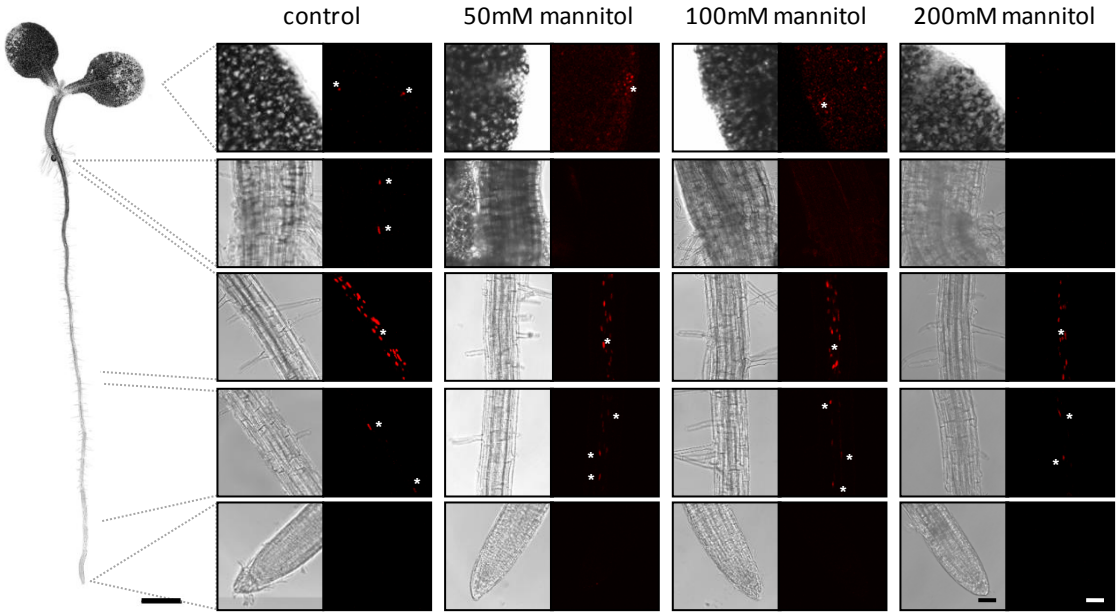
| purpose | DNA-sequence |
|---|---|
| AHK1-ED Leu298Ala | TATGGATTCTCCGCTG**GCC**AGTGCGGCCCTGCCGG |
| | CCGGCAGGGCCGCACT**GGC**CAGCGGAGAATCCATA |
| AHK1-ED Leu422Ala | CCTGAATCTGAAACG**GGCG**CCGATCGTTGGCGTTGTCA |
| | TGACAACGCCAACGATCGG**CGCC**CGTTTCAGATTCAGG |
| MKKK20 insert stop | TGGGAGTTGGCTTACAGTCCGG**TGA**AAGGGTGGGCGCGCCGACCCAGCTTTCTTG |
| | CAAGAAAGCTGGGTCGGCGCGCCCACCCTT**TCA**CCGGACTGTAAGCCAACTCCCA |
| IAA16 remove stop | GTGCAAGAACAGAAGT**TTA**AAGGGTGGGCGCGCCGA |
| | TCGGCGCGCCCACCCTT**TAA**ACTTCTGTTCTTGCAC |
| IAA16 Ser150Ala | TCTCTCCAACGCCTTA**GCC**AAAATGTTTAGC |
| | GCTAAACATTTT**GGC**TAAGGCGTTGGAGAGA |
| IAA16 Ser150Glu | TCTCTCCAACGCCTTA**GAG**AAAATGTTTAGCTC |
| | GAGCTAAACATTTT**CTC**TAAGGCGTTGGAGAGA |
| WAK4 remove stop | CTGGACATTGAAGCAGGCCGC**AAG**GGTGGGCGCGCCGACCCAGCTTTCTTG |
| | CAAGAAAGCTGGGTCGGCGCGCCCACC**CTT**GCGGCCTGCTTCAATGTCCAG |
| BRI1 Ser1172Ala | CAGTCAACGATCAGA**GCA**ATAGAGGATGGAGGGTTCAG |
| | CTGAACCCTCCATCCTCTAT**TGC**TCTGATCGTTGACTG |
| BRI1 Ser1172Glu | CAGTCAACGATCAGA**GAA**ATAGAGGATGGAGGGTTCAG |
| | CTGAACCCTCCATCCTCTAT**TTC**TCTGATCGTTGACTG |

## A9: Oligonucleotides for sequencing by GATC

| purpose | DNA-sequence |
|---|---|
| intern AHK1 primer for complete sequencing (fwd1) | AGGGCCTGAAGATGTAAG |
| intern AHK1 primer for complete sequencing (fwd2) | AGAGACTTCCAATTGTAGGTGT |
| intern AHK1 primer for complete sequencing (fwd3) | TGCCAGCATTAGTCAGAG |
| intern AHK1 primer for complete sequencing (fwd4) | TATGGAAGTACAGCAAGAATGAT |
| sequencing of insert in *pMetYC-Dest* (fwd) | AGGGTCGTCAGATACATAGA |
| sequencing of insert in *pMetYC-Dest* (rev) | TTGTCCACGGCTTCATCGTG |
| sequencing of insert in *pMetYC-Dest* (fwd) | ATTTCAAGCTATACCAAGCA |
| sequencing of insert in *pMetYC-Dest* (rev) | CAGCGTAATCTGGAACGTCA |
| sequencing of insert in *pB7WGR2* (rev) | TAGATTTGTAGAGAGAGACTGG |
| sequencing of insert in *pB7WGR2* (fwd) | GGACTACACCATCGTGGAACAG |
| intern At1G14390 primer for complete sequencing (fwd) | CTCCTTCTGCAAAGTATCAGCG |
| intern At1g72250 primer for complete sequencing (fwd1) | AGTATTACAACTGATGCTG |
| intern At1g72250 primer for complete sequencing (fwd2) | AGTCCAAGATATCAACGAG |
| intern At1g72250 primer for complete sequencing (fwd3) | ACGGCTAATGAGCACAG |
| intern At1g72250 primer for complete sequencing (fwd4) | AAGCTTGCTCGTCAACATG |
| intern WAK4 primer for complete sequencing (fwd1) | ATGAAGCAAATGGAGAATGTAA |
| intern WAK4 primer for complete sequencing (fwd2) | TGCCATTAGCTGTATAGAACA |
| intern WAK4 primer for complete sequencing (fwd3) | GGTCTATGAGTTCATTTCCAG |

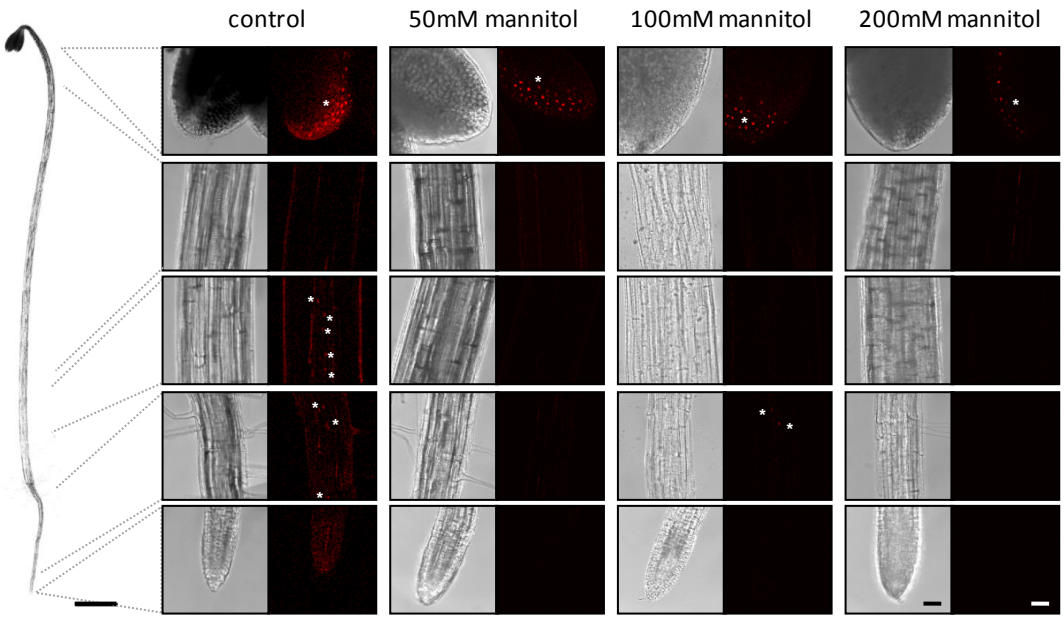
| purpose | DNA-sequence |
|---|---|
| intern AHA2 primer for complete sequencing (fwd1) | CTGCAAACAAGGAGAAATCG |
| intern AHA2 primer for complete sequencing (fwd2) | TGGAATCAGGGAAGTTCAC |
| intern AHA2 primer for complete sequencing (fwd3) | TTGCTGATGCTACAGATGC |
| intern AHA2 primer for complete sequencing (fwd4) | TTGCACAACTGATTGCTAC |

## A10: *AHK1$_{pro}$* activity in mannitol-treated light-grown seedlings



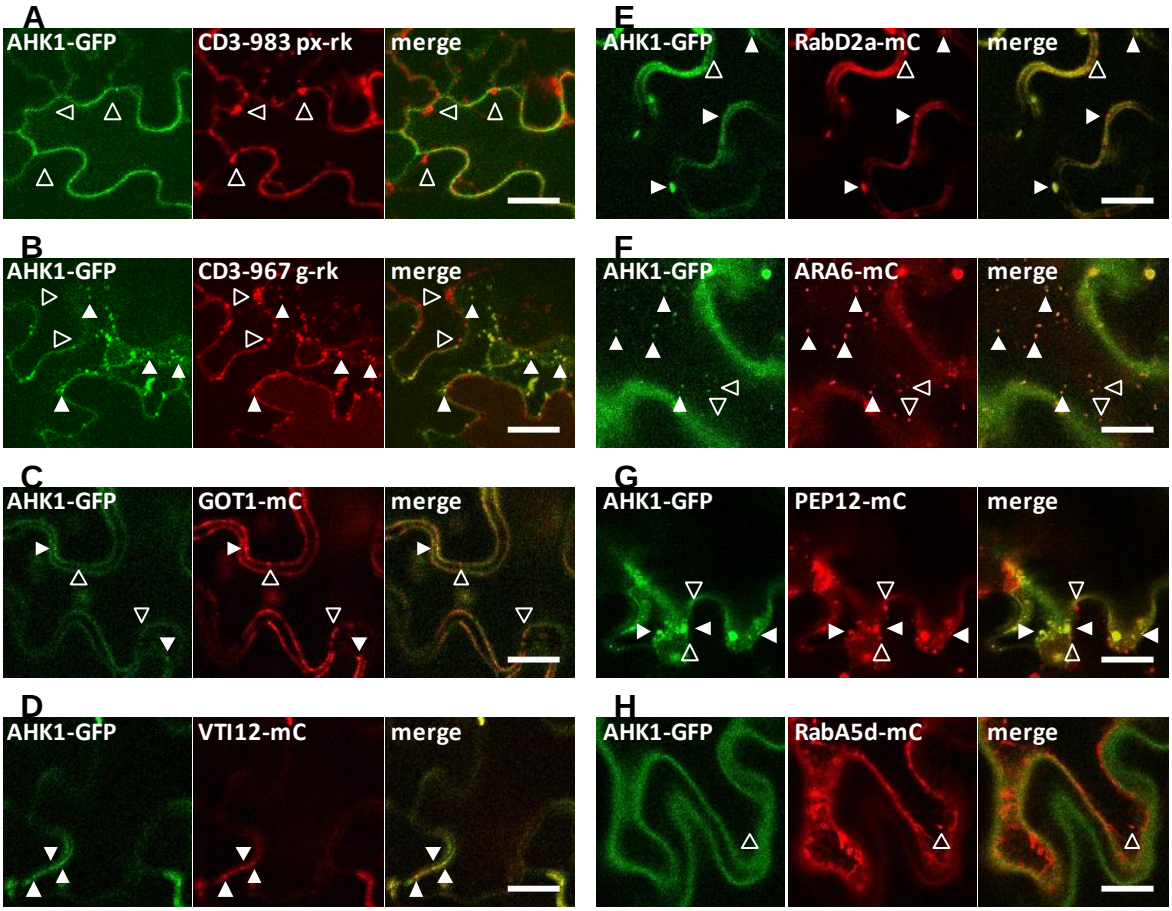

To analyze the tissue specificity of the *AHK1-promoter* (*AHK1$_{pro}$*) it was fused to *mCherry* (red) and a *nuclear localization signal* (*NLS*) in a binary plant vector (*pB7-AHK1pro-mCherryNLS*) and stably transformed into *Arabidopsis thaliana* Col-0. The tissue specificity of *AHK1$_{pro}$* activity and *mCherry* expression was analyzed in three day old light grown seedlings with confokal microscopy. Different laser intensities were used for the detection of mCherryNLS. Shown is a brightfield overview image of the seedling as well as close ups which show brightfield images and mCherry signal (red). Dashed lines mark the zone of the seedling for which the shown pictures are representative. At least six seedlings per treatment were investigated. The bars in the overviews give 1mm, the bars in the close ups 0.05mm. Stars mark nucleic mCherry signal.

## A11: *AHK1$_{pro}$* activity in mannitol-treated etiolated seedlings



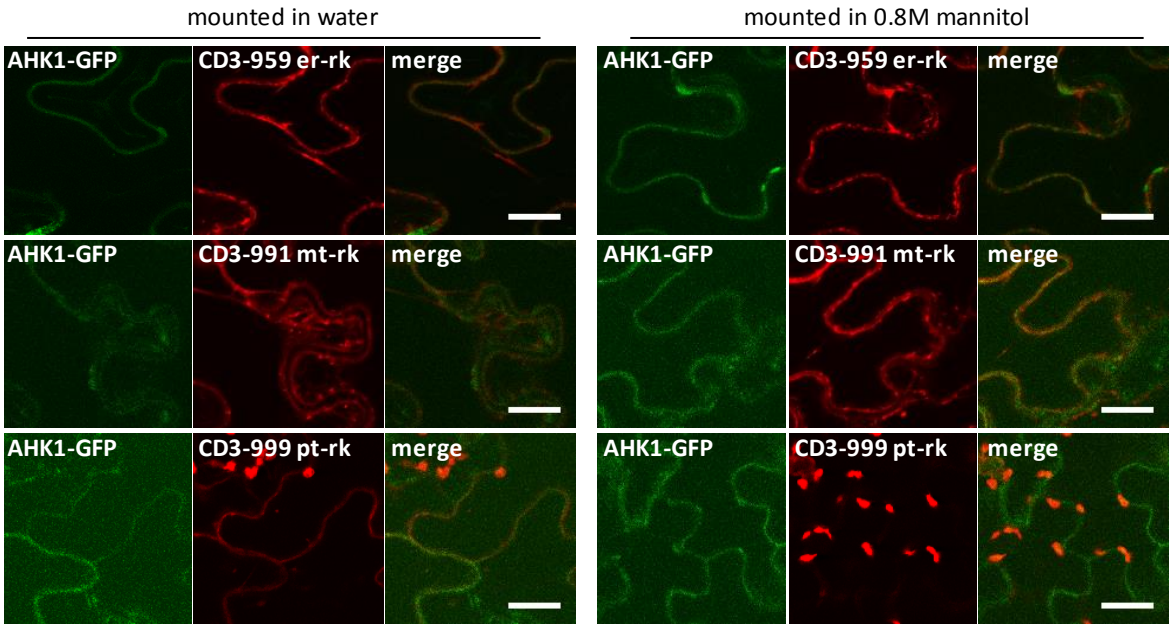

To analyze the tissue specificity of the *AHK1-promoter* (*AHK1$_{pro}$*) it was fused to *mCherry* (red) and a *nuclear localization signal* (*NLS*) in a binary plant vector (*pB7-AHK1pro-mCherryNLS*) and stably transformed into *Arabidopsis thaliana* Col-0. The tissue specificity of *AHK1$_{pro}$* activity and *mCherry* expression was analyzed in three day old etiolated seedlings with confokal microscopy. Different laser intensities were used for the detection of mCherryNLS. Shown is a brightfield overview image of the seedling as well as close ups which show brightfield images and mCherry signal (red). Dashed lines mark the zone of the seedling for which the shown pictures are representative. At least six seedlings per treatment were investigated. The bars in the overviews give 1mm, the bars in the close ups 0.05mm. Stars mark nucleic mCherry signal.

## A12: Subcellular localization of AHK1 in N. benthamiana treated with 0.8M mannitol



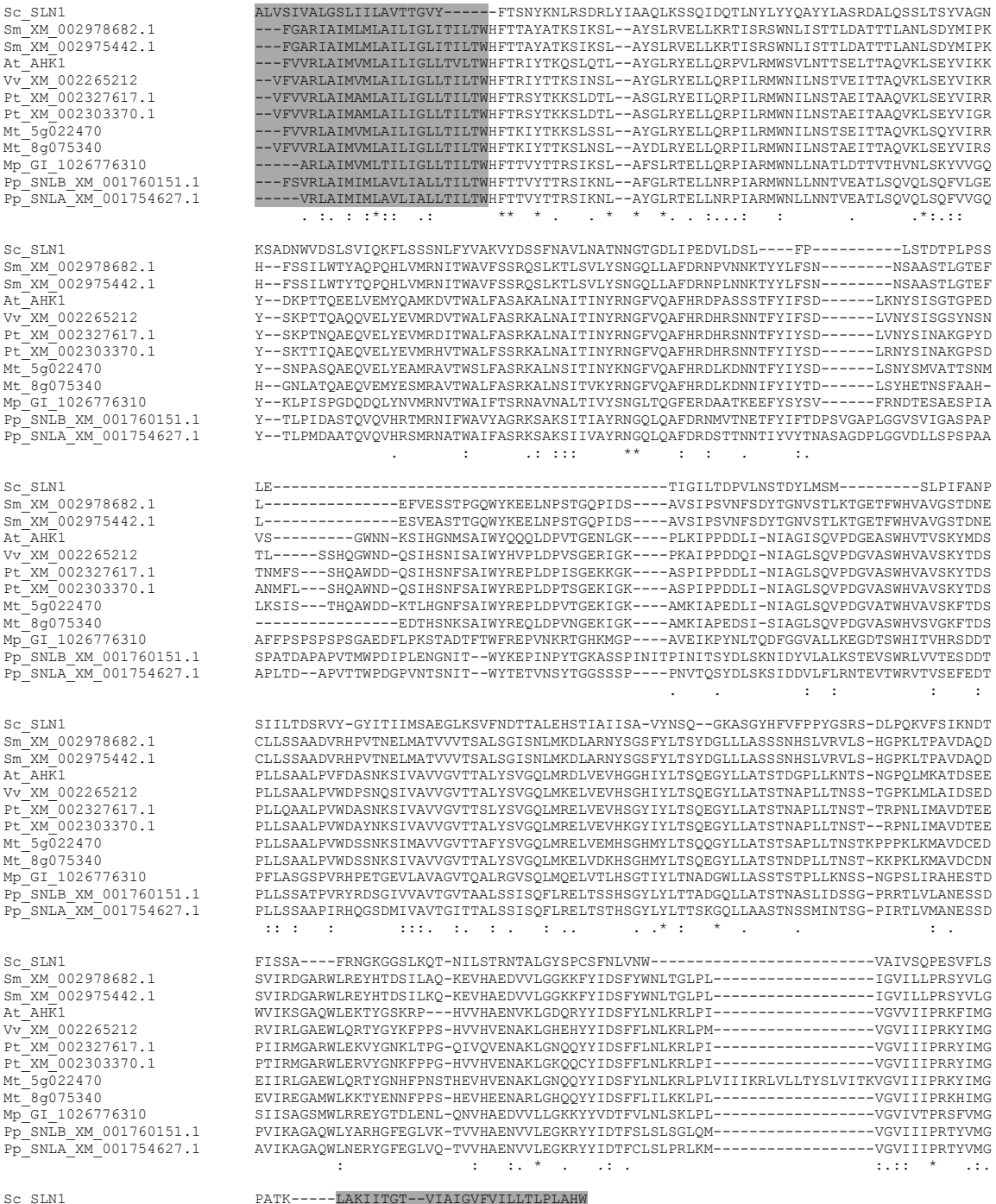

Transient co-expression of *AHK1-GFP* with (A) the peroxisomal marker *CD3-983 px-rk*; (B) the golgi-marker *CD3-967 g-rk*; (C) the golgi-marker *GOT1-mC*; (D) *VTI12-mC* as marker for the trans-golgi-network and early endosomes; (E) *RabD2a-mC* as marker for endosomes and the golgi-system; (F) *ARA6-mC* as marker for late endosomes and prevacuolar compartments; (G) *PEP12-mC* as marker for the post golgi compartment and with (H) *RabA5d-mC* as marker for the recycling endosome. (A, B) were obtained from Andreas Nebenführ (Nelson *et al.*, 2007). The markers are fused with *RFP* and under the control of the *35S-promoter*. *AHK1-GFP* was encoded in *pH7FWG2.0-AHK1* and expressed under control of the *35S-promoter* as well. (C-H) The markers for organelles fused with *mCherry* (mC) were obtained from Peter Pimpl who used the β-estradiol inducible promoter *lexA$^{-46-}$35S* (Zuo *et al.* 2000). These marker constructs were co-transformed into *N. benthamiana* together with a vector in which *AHK1-GFP* is under control of *lexA$^{-46}$35S* as well. The subcellular localization was analyzed two days after transformation (A-H) and 4-24h after β-estradiol induction (C-H). Thereby the tobacco leaves were mounted in 0.8M mannitol and incubated for at least 10min. White outlined arrows mark vesicle-like structures which do not show co-localization, white arrows mark co-localization. The scale in all images is 20µm.

## A13: Transient co-expression of AHK1-GFP with markers for the endoplasmic reticulum, mitochondria and plastids in N. benthamiana




Transient expression of *AHK1-GFP* with markers for the endoplasmatic reticulum (*CD3959 er-rk*), mitochondria (*CD3-991 mt-rk*) and plastids (*CD3-999 pt-rk*). The markers were obtained from Andreas Nebenführ (Nelson *et al.*, 2007), fused with *RFP* and under the control of the *35S-promoter*. These marker constructs were co-transformed into *N. benthamiana* with *pH7FWG2.0-AHK1*, a vector in which *AHK1-GFP* is under control of the *35S-promoter* as well. The subcellular localization was analyzed two days after transformation. Thereby the tobacco leaves were mounted in water or 0.8M mannitol and incubated for at least 10min. The scale in all images is 20µm.

## A14: Amino acid sequence alignment of SLN1-ED, AHK1-ED and AHK1-ED similar sequences



CLUSTAL multiple sequence alignment by MUSCLE (3.8) of the amino acid sequences of the extracellular domain (ED) of *Saccharomyces cerevisiae* (Sc) SLN1 (SLN1-ED; aa25-356), *Arabidopsis thaliana* (At) AHK1 (AHK1-ED; aa100-446) and similar sequences from *Selaginella moellendorffii* (Sm), *Physcomitrella patens* (Pp), *Marchantia polymorpha* (Mp), *Vitis vinifera* (Vv), *Medicago truncatula* (Mt) and *Populus trichocarpa* (Pt). Predicted transmembrane domains (Dinkel *et al.*, 2016; AHK1-ED: aa77-99; aa447-469; dark grey) were included in the alignment. Identical residues are highlighted with "*". Highly conserved residues are designated with ":", weakly conserved residues with ".".

## A15: Amino acid sequence alignment of SLN1-ED and CKI1-ED

```
Sc SLN1                 ALVSIVALGSLIILAVTTGVYFTSNYK----NLRSDRLYIAAQLKSSQID--QTLNYLYYQAYYLASRDALQSSLTSYVAGNKSA
At_CKI1_NM_130311.2     ASRPIVVFCVLAFLVVVFECIWISNWRTTTENLVKEVASFTEDLRTSLVSEIENIGKFTYAKTNLSTI-GLARVIDSYITNNDTG
                        *  .**.:  * :*.*.    : **:.    ** .:   :: :*.:* :.  :.:. : *    *::  .*   : **::.*.:.

Sc_SLN1                 DNWVDSLSVIQKFLSSSNLFYVAKVYDSSFNAVL----------------NATNNGTGDLIP-EDVLDSLF-PLSTDTPLPSSLE
At_CKI1 NM 130311.2      FTEIQTQIAPLLFVAYSTILQVSQVSYISRDGLMFSYIAESNTSVAVFANSSSNSSRGDYTWYTQTVDQLTGRLNGNSTKSQSLD
                         . :::  .   *:: *.:: *::*   * :.::                .::*.. **     :.:*.*   *. ::. ..**:

Sc SLN1                 TIGILTDPVLNSTDY---LMSMSLPIFANPSIILTDSRVY---GYITIIMSAEGLKSVFNDTTALEHSTIAIIS---AVYNSQGK
At_CKI1_NM_130311.2     VTHTDWFQAAQSNNYTTAFVGTSLGGEDNETLIQSVVSLYSKKGLVSLGFPVKTLTEVLN-SLNLHGEELYMWTKDGTVLVREGS
                        .       . :*.:*   ::. **    * ::* :   :*   * ::: :..: *..*:* :  *  . : : :   :*   :*.

Sc SLN1                 ASGYHFVFPPYGSRSDLPQKVFSIKNDTFISSAFRNGKGGSLKQTNILSTRNTALGYSPCSFNLVNWVAIVSQPESVFLSPATKL
At_CKI1_NM_130311.2     LNDSFFI-----SNGSI---CFGRESNSLWSQCIPENCSSSGYEVEIKRLRYQAF----CSVIEVSGVPLRYTLMFPNKGGATRI
                         .. .*:     *...:    *. :.::: *..: :. ..*  :.:*   *  *:    **.  *. *.:         . **.:

Sc SLN1                 AKIITGTVIAIGVFVILLTLPLAHW------
At_CKI1_NM_130311.2     KHQAEKAKYQLIVVMIFLGFGWPVWFVWFMM
                         :    :   : *.:*:* :  . *
```

CLUSTAL multiple sequence alignment by MUSCLE (3.8) of the amino acid sequences of the extracellular domain (ED) of *Saccharomyces cerevisiae* (Sc) SLN1 (SLN1-ED; aa25-356) and *Arabidopsis thaliana* (At) CKI1 (CKI1-ED; aa10-366). Predicted transmembrane domains (Dinkel *et al.*, 2016; dark grey) were included in the alignment. Identical residues are highlighted with "*". Highly conserved residues are designated with ":", weakly conserved residues with ".".

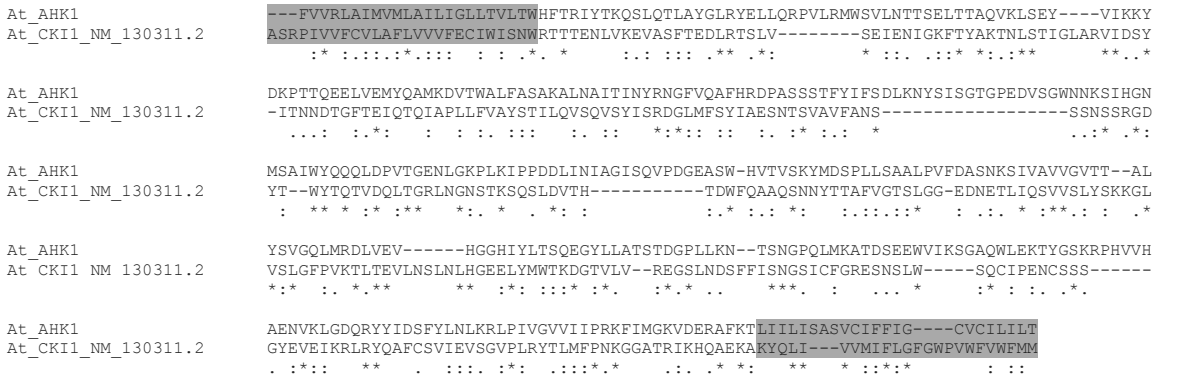
## A16: Amino acid sequence alignment of SLN1-ED and AHK1-ED

```
At AHK1         ---FVVRLAIMVMLAILIGLLTVLTWHFTRIYTKQSLQTL--AYGLRYELLQRPVLRMWSVLNTTSELTTAQVKLSEYVIKKYDKPTTQEELVE
Sc_SLN1         ALVSIVALGSLIILAVTTGV------YFTSNYKNLRSDRLYIAAQLKSSQIDQTLNYLYYQAYYLASRDALQSSLTSYVAGN----KSADNWVD
                    :* *. :::**:  *:      :**  *.:   : *  *  *. . ::..:  ::      :.  : * .*:.**  :    .: :: *:

At_AHK1         MYQAMKDVTWALFASAKALNAITINYRNGFVQAFHRDPASSSTFYIFSDLKNYSISGTG---PEDVSGWNNKSIHGNMSAIWYQQQLDPVTGEN
Sc_SLN1         SLSVIQK-----FLSSSNLFYVAKVYDSSF----------------NAVLNATNNGTGDLIPEDV--------------------LDSLFPLS
                  ..::.     * *:. *  ::  * ..*                 . : * : .***   ****                    **.:   .

At_AHK1         LGKPLKIPPDDLINIAGISQVPDGEASWHVTVSKYMDSPLLSAALPVFDA-----SNKSIVAVVGVTTALYSVGQLMRDLVEVHGGHIYLTS--
Sc_SLN1         TDTPL---PSSLETI-GILTDP------VLNSTDY----LMSMSLPIFANPSIILTDSRVYGYITIIMSAEGLKSVFNDTTALEHSTIAIISAV
                 ..**   *..* .* **   *       :. :.*    *:* :**:*       ::. : . : :  :  .: .::.* . :  . * : *

At_AHK1         ------QEGYLLATSTDGPLLKNTSNGPQLMKATDSEEWVIKSGAQWLEKTYGSKRPHVVHAENVKLGDQRYYIDSFYLNLKRLPIVGVVIIPR
Sc_SLN1         YNSQGKASGYHFVF----PPYGSRSDLPQKVFSIKNDTFI--SSAFRNGKGGSLKQTNILSTRNTALG---YSPCSFNL----VNWVAIVSQPE
                      .** :.     *   . *: ** : : ..: ::  *.* .  *  . *..::: : *. **   *   ** *    :  *.:*  *.

At_AHK1         KFIMGKVDERAFKTLIILISASVCIFFIGCVCILILT-----
Sc SLN1         SVFLSPATK-----LAKIITGTVIA--IGVFVILLTLPLAHW
                .::. . :     *  :*:.:*    ** . **:
```

CLUSTAL multiple sequence alignment by MUSCLE (3.8) of the amino acid sequences of the extracellular domain (ED) of *Saccharomyces cerevisiae* (Sc) SLN1 (SLN1-ED; aa25-356) and *Arabidopsis thaliana* (At) AHK1 (AHK1-ED; aa100-446). Predicted transmembrane domains (Dinkel

*et al.*, 2016; dark grey) were included in the alignment. Identical residues are highlighted with "*". Highly conserved residues are designated with ":", weakly conserved residues with ".".

## A17: Amino acid sequence alignment of AHK1-ED and CKI1-ED

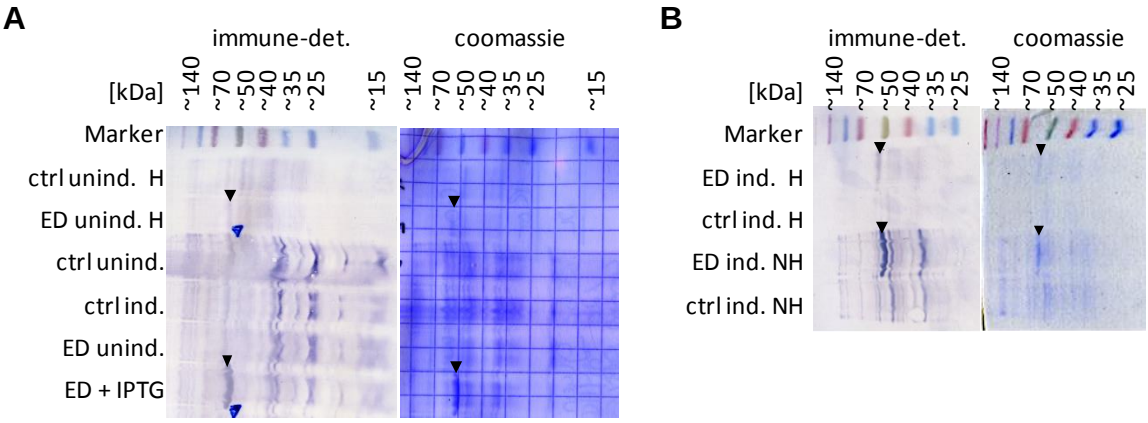



CLUSTAL multiple sequence alignment by MUSCLE (3.8) of the amino acid sequences of the extracellular domain (ED) of *Arabidopsis thaliana* (At) AHK1 (AHK1-ED; aa100-446) and CKI1 (CKI1-ED; aa10-366). Predicted transmembrane domains (Dinkel *et al.*, 2016; dark grey) were included in the alignment. Identical residues are highlighted with "*". Highly conserved residues are designated with ":", weakly conserved residues with ".".

## A18: Amino acid sequence alignment of AHK1-ED, SLN1-ED and CKI1-ED

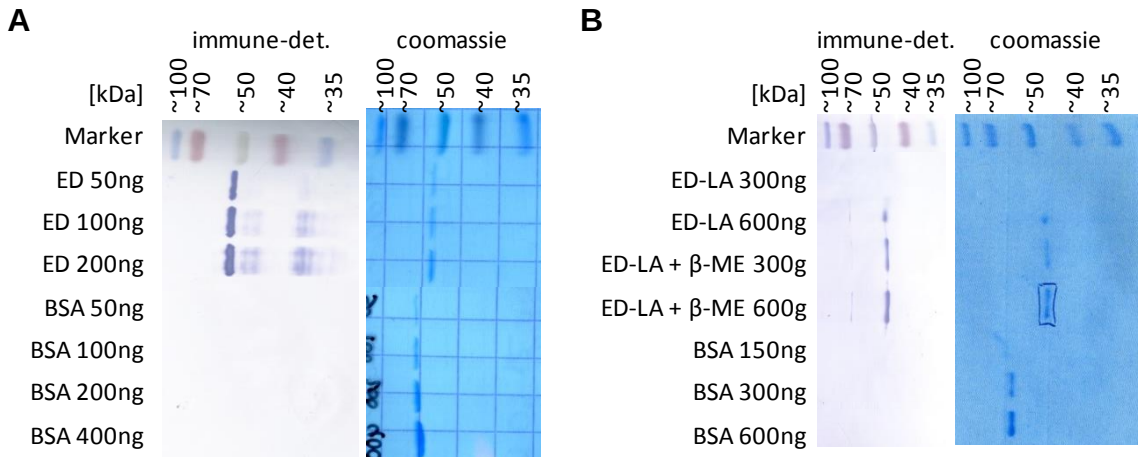

CLUSTAL multiple sequence alignment by MUSCLE (3.8) of the amino acid sequences of the extracellular domain (ED) of *Saccharomyces cerevisiae* (Sc) SLN1 (SLN1-ED; aa25-356); *Arabidopsis thaliana* (At) AHK1 (AHK1-ED; aa100-446) and CKI1 (CKI1-ED; aa10-366). Predicted transmembrane domains (Dinkel *et al.*, 2016; dark grey) were included in the alignment. Identical residues are highlighted with "*". Highly conserved residues are designated with ":", weakly conserved residues with ".".

## A19: Detection of AHK1-ED in protein extract from transformed Origami2 (DE3) cells




(A) Immune-detection and SDS-gel which was stained with Coomassie revealing abundance of AHK1-ED (black arrows) in Origami2 (DE3) cells which were transformed with *pMH-Hssumo-AHK1-ED* 26h after induction of protein expression with 0.3M IPTG (ind.) and without IPTG-induction (unind.). Untransformed Origami2 (DE3) cells were used as control (ctrl). AHK1-ED was detected with the use of α-His-AP. (B) Comparison of samples which were heated (H) and not heated (NH) for 10min to 95°C after the addition of Lyse and Load (LL)-buffer to the cells for protein extraction. Cells were lysed in the volume of LL-buffer which would have been needed to set $OD_{600}$=4.0. 25µl of protein extract were loaded. Spectra™ Multicolor Broad Range Protein Ladder was used as size marker.

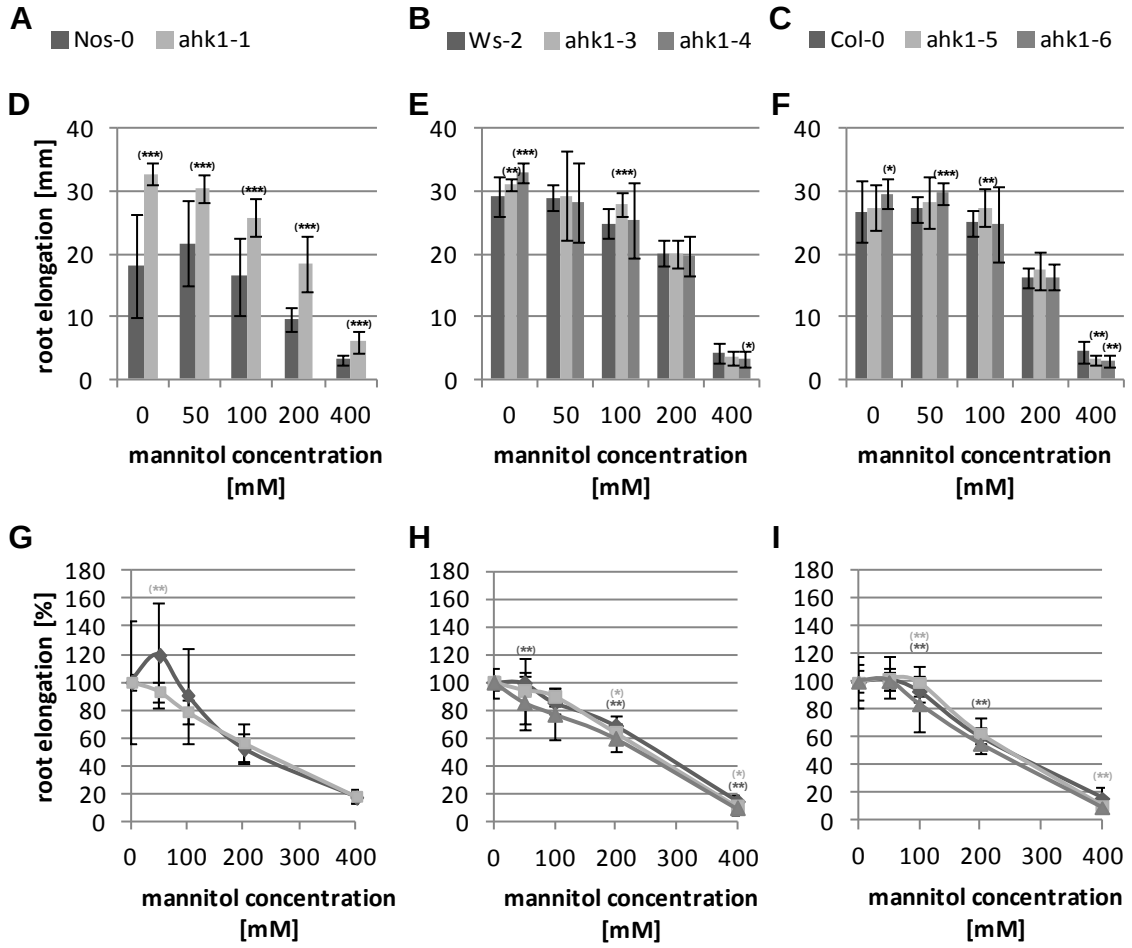
## A20: AHK1-ED and AHK1-ED-Leu298/422Ala expressed by gene cust




Immune-detection and SDS-gel which was stained with Coomassie revealing abundance of AHK1-ED (A; ED) and AHK1-ED-Leu298/422Ala (B; ED-LA) which was expressed and purified by GeneCust. Bovine serum albumin (BSA) was used as quantity control. β-mercaptoethanol (β-ME) was used as

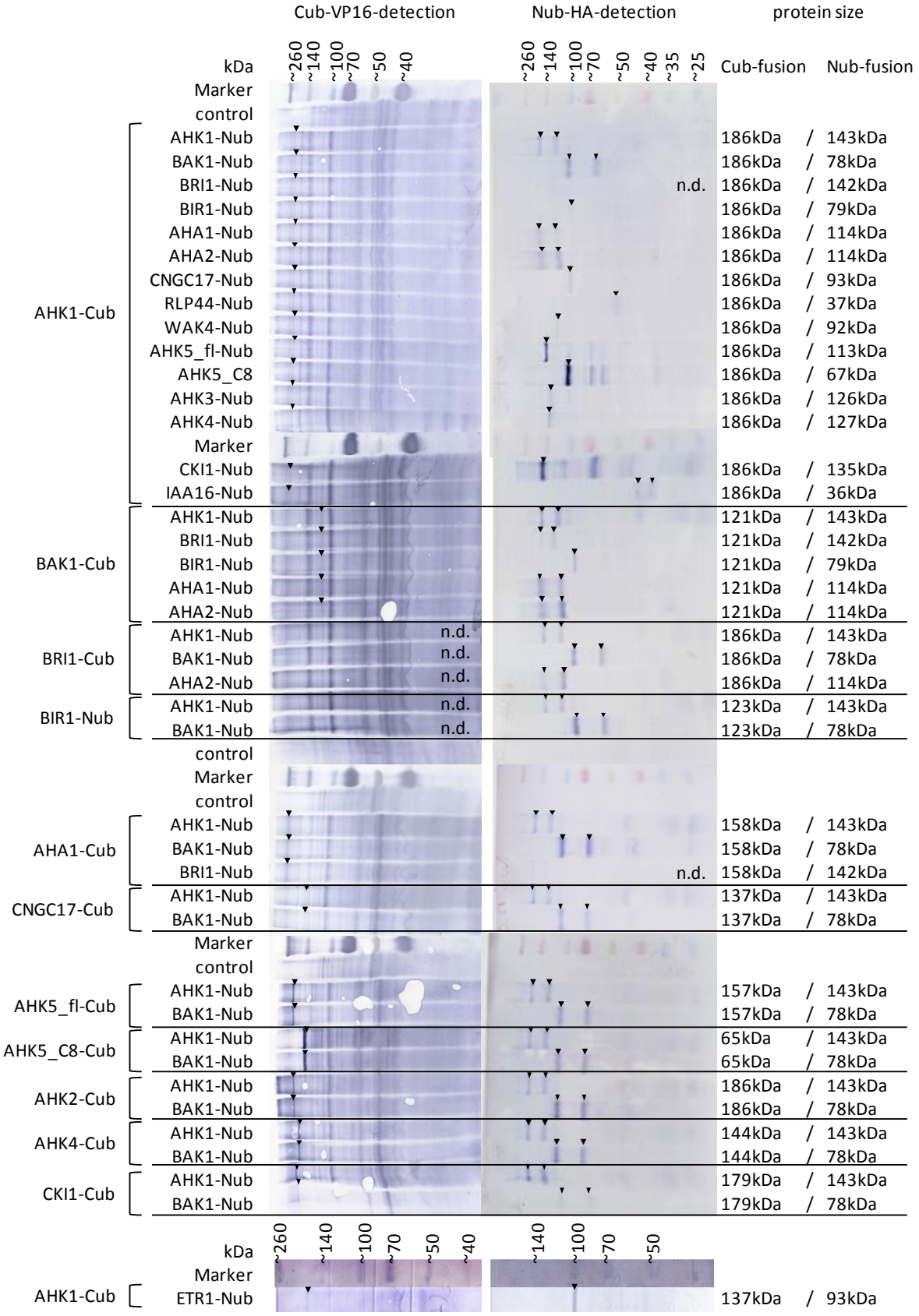
reducing agent for AHK1-ED-Leu298/422Ala. AHK1-ED and AHK1-ED-Leu298/422Ala were detected with the use of α-His-AP. Spectra™ Multicolor Broad Range Protein Ladder was used as size marker.

## A21: Root elongation of *ahk1* knock down lines in different ecotypes during mannitol stress



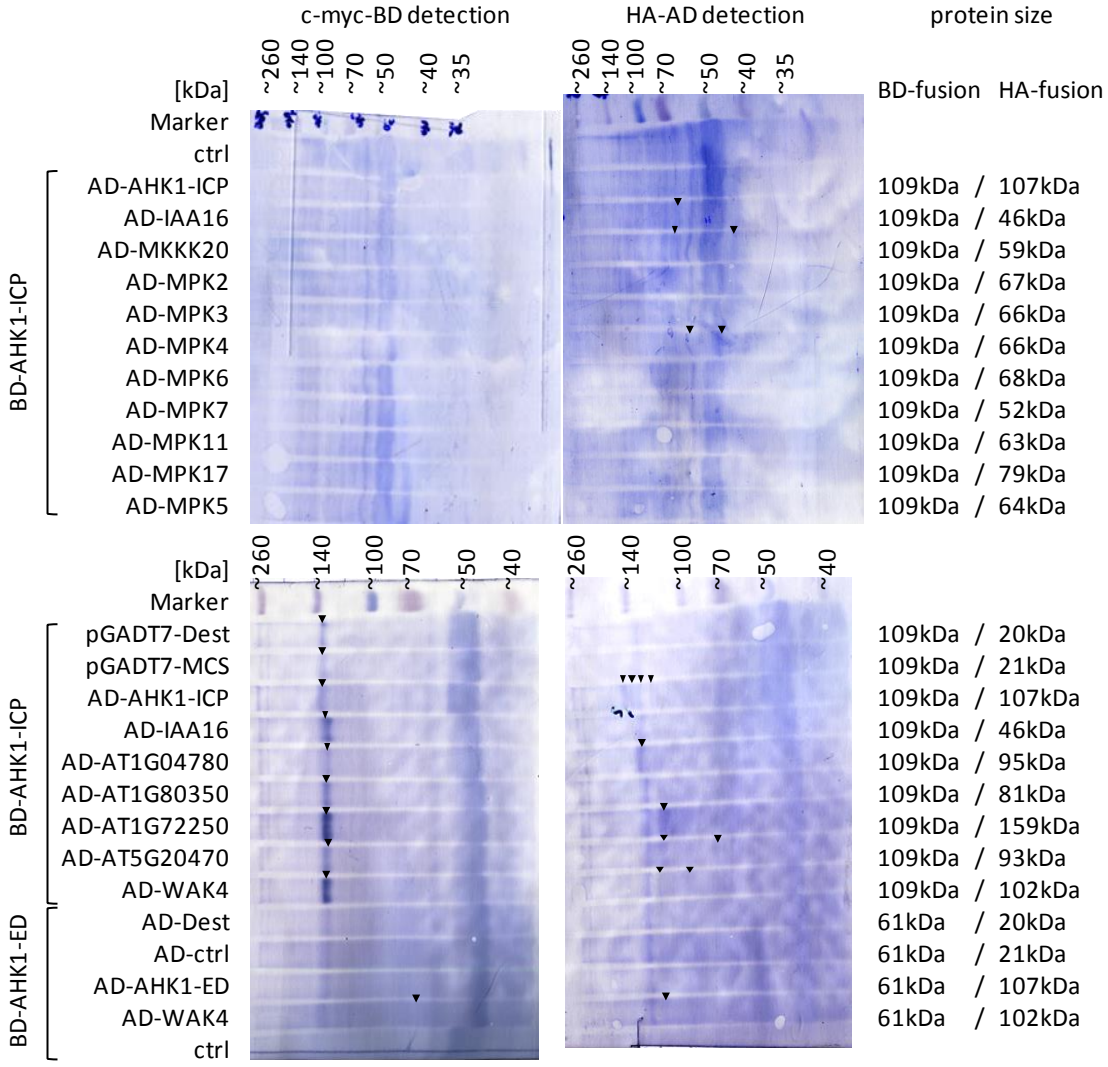

The *ahk1* knock down alleles in the Nos-0 (A, D, G), Ws-2 (B, E, H) and Col-0 (C, F, I) ecotype were grown for four days on half strength MS salts at constant light conditions, were then transferred to sorbitol-supplemented media and grown for additional four days. The root elongation was analyzed with the method of Kumar *et al.* (2013) showing the total root elongation (D, E, F) and the method of Wohlbach *et al.* (2008) showing the mean percentage of root elongation based on a non-stressed control root (G, H, I). (A) gives the color code for (D) and (G), (B) gives the color code for (E) and (H), (C) gives the color code for (F) and (I). The labeling of the y-axis in (D) is also valid for the y-axes in (E) and (F) the labeling of the y-axis in (G) is also valid for the y-axes in (H) and (I). Shown are mean values and standard deviations of one experiment with at least 20 seedlings per line and treatment. Student‘s t-test was used to analyze statistical significance of differences. Stars above the bars display statistical significance in comparison to the respective wildtype. *p<0.05; **p<0.01; ***p<0.001. Brackets around stars display that the significance has been shown in just one experiment.

## A22: Immunodetection of protein expression for mating-based split-ubiquitin screens



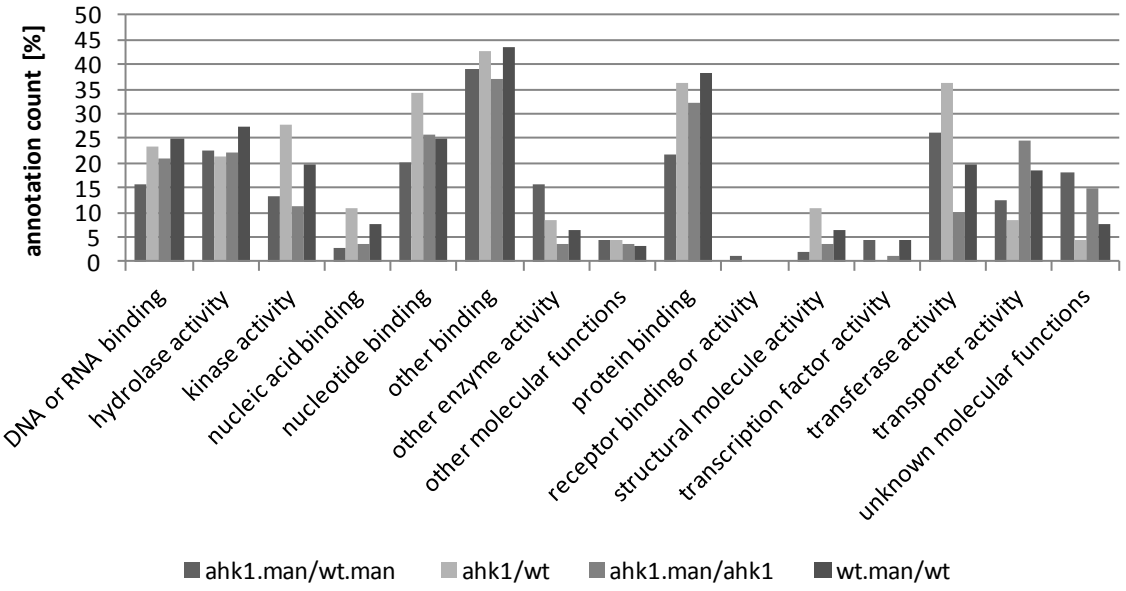
The Cub-fusions were detected with the use of α-VP16 (rabbit) and α-rabbit-AP, the Nub-fusions were detected with α-HA (rat) and α-rat-AP. SpectraTM Multicolor Broad Range Protein Ladder was used as size marker. Black arrows highlight detected fusion proteins. Not detected proteins are marked with „n.d.“. Untransformed THY.AP4 and THY.AP5 yeast strains were used as control.

## A23: Immunodetection of protein expression for yeast-two-hybrid screens



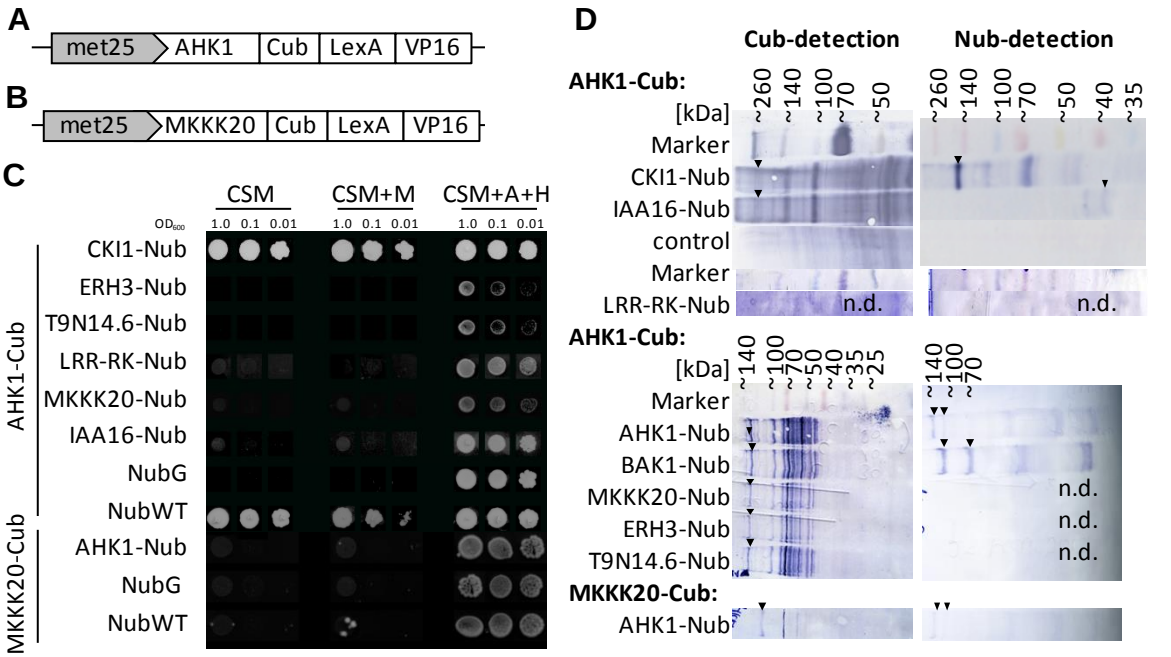

The intracellular part (ICP) of AHK1 (AHK1-ICP) and the extracellular domain (ED) of AHK1 (AHK1-ED) which were fused with the binding domain (BD) and the ten amino acid comprising c-myc-tag encoded in the respective vector *pGBKT7* were detected with the antibodies α-c-myc (mouse) and α-mouse-AP, the proteins which were fused with the activating domain (AD) and the nine amino acid comprising HA-tag which was derived from the Human influenza hemagglutinin glycoprotein and which were encoded in the respective vector *pGADT7* were detected with the antibodies α-HA (rat) and α-rat-AP. Spectra™ Multicolor Broad Range Protein Ladder was used as size marker. Black arrows highlight detected fusion proteins. Untransformed *Saccharomyces cereviasiae* pJ69-4A was used as control (ctrl).

## A24: Gene ontology annotation for the molecular function of the quantified phosphopeptides



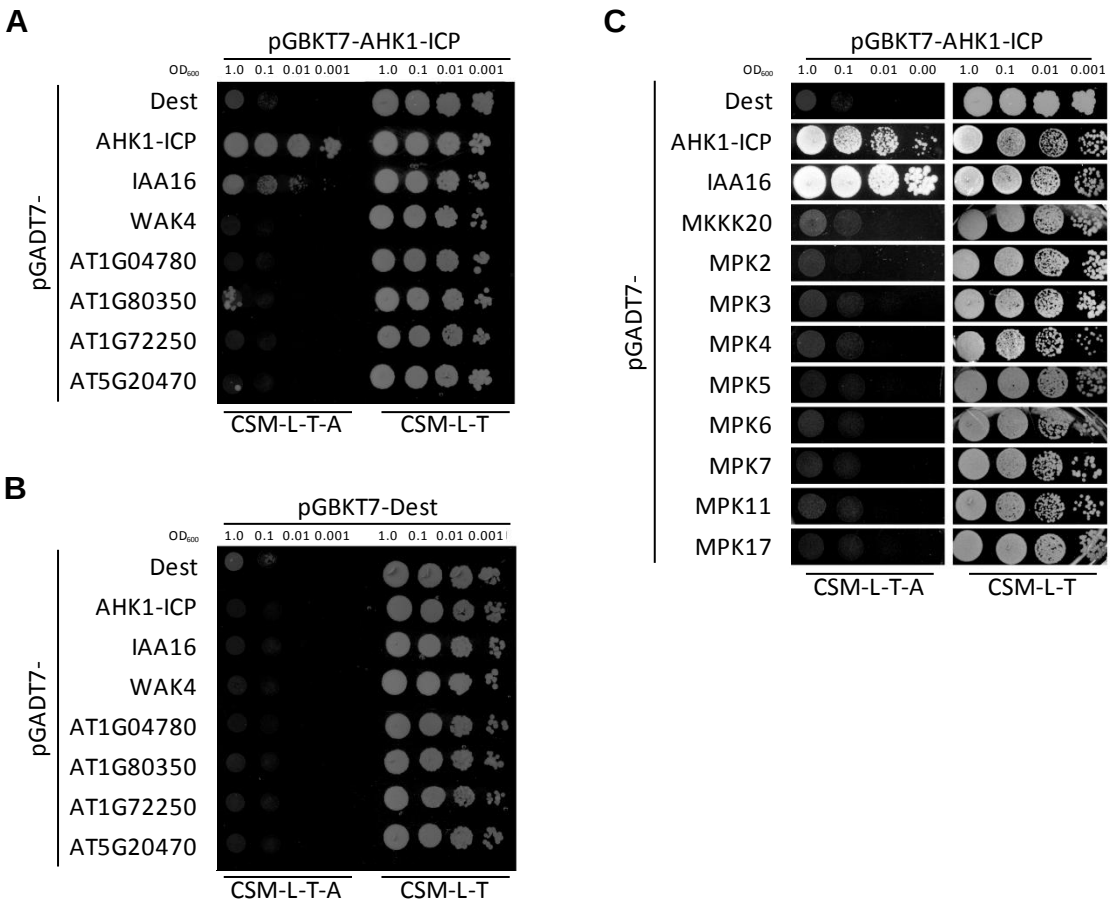

Gene ontology annotation for the molecular function of the quantified phosphopeptides in *ahk1-3* (ahk1) and the wildtype (wt) Ws-2 after 10min treatment with 0.3M mannitol (ahk1.man; wt.man) or mock (ahk1; wt). ahk1.man/wt.man and ahk1/wt comprise phosphopeptides which were quantified in the experiment with and without metabolic labeling, ahk1.man/ahk1 and wt.man/wt comprise phosphopeptides which were quantified in the experiment without metabolic labeling.

## A25: Mating-based split-ubiquitin assay to test the interaction of AHK1 with cytoskeleton-associated proteins, MKKK20 and IAA16



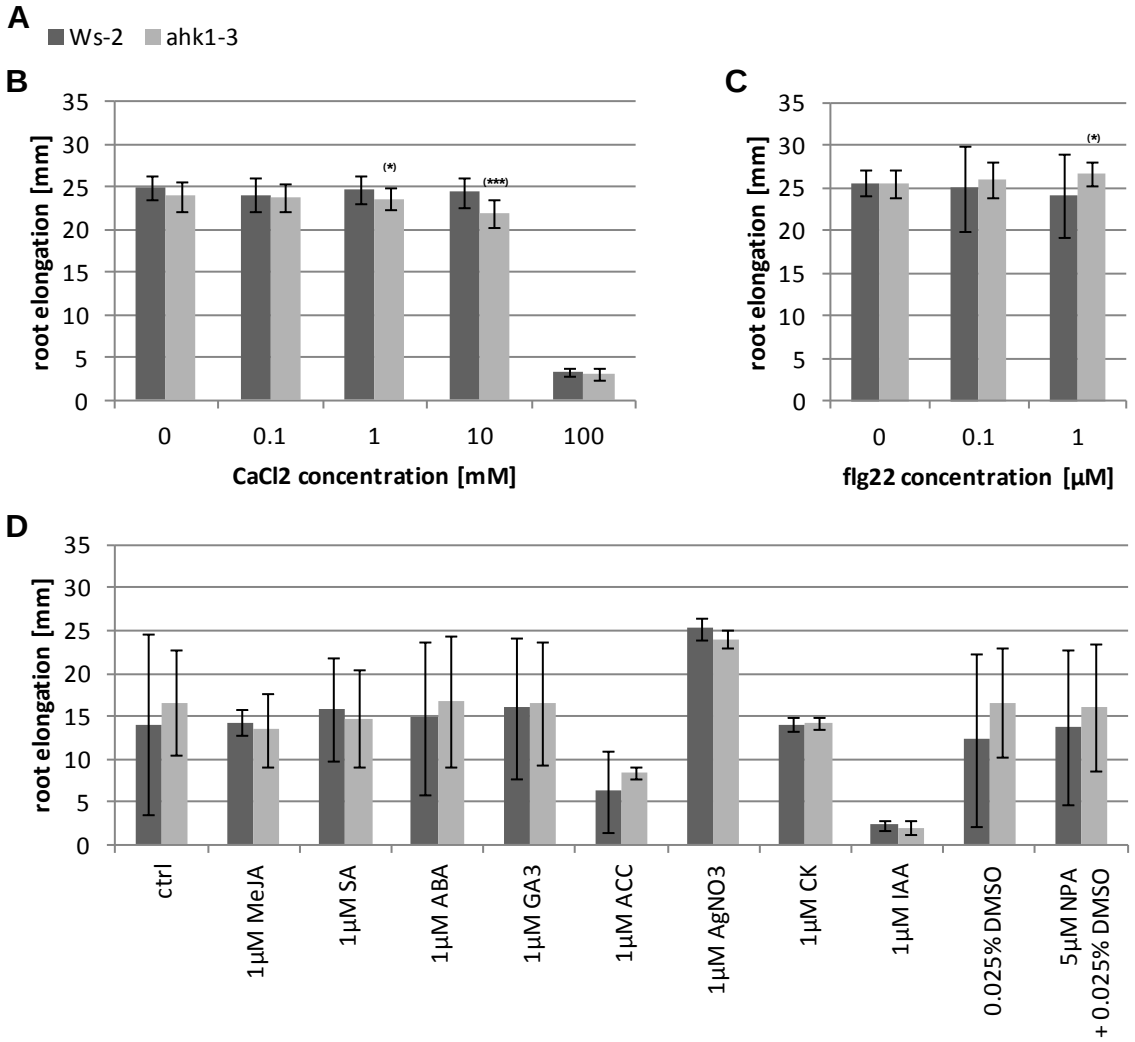

The fusion constructs of *AHK1* (A) and *MKKK20* (B) with the carboxy terminal part of *ubiquitin* (Cub) including the tags for expression activation (LexA, VP16) in the *pMetYC*-Dest vector were transformed into the *S. cerevisiae* strain THY.AP4, fusion constructs of *CKI1*, *ERH3* (*AT1G80350*), *T914.6* (*AT1G72250*), *LRR-RK* (*AT1G14390*), *MKKK20* and *IAA16* with the amino terminal part of *ubiquitin* (*Nub*) in the *pXNubA22-Dest* vector as well as the negative control *NubG* and the positive control *NubWT* were transformed into the *S. cerevisiae* strain THY. AP5. After the mating the interaction of the proteins was tested by dropping of the yeast on CSM minimal medium (CSM) and verified by dropping them on CSM supplemented with 50µM Met (CSM+M) whereas CSM-Ade+-His+ (CSM+A+H) served as growth control. The growth was recorded after 4d growth at 28°C (C). (D) The Cub-fusion proteins were detected with α-VP16 (rabbit) and α-rabbit-AP, the Nub-fusion proteins were detected with α-HA (rat) and α-rat-AP. Spectra™ Multicolor Broad Range Protein Ladder was used as size marker. Black arrows highlight the detected protein. Not detected protein is marked with "n.d.". Untransformed THY.AP4 and THY.AP5 were used as control.

## A26: Yeast-two-hybrid screen to test the interaction of AHK1-ICP with cytoskeleton-associated proteins, MAP-kinases, IAA16 and WAK4



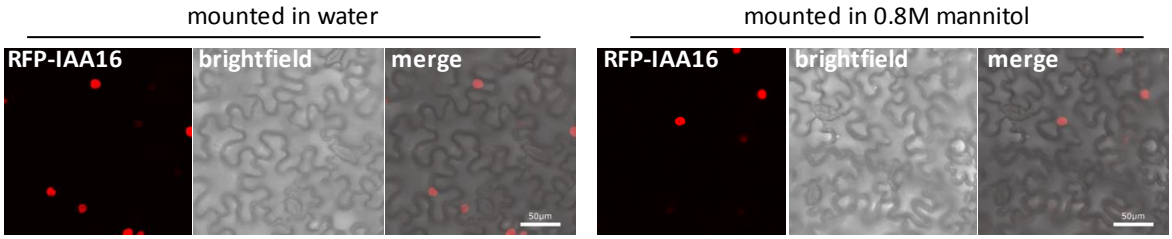

The fusion construct of *AHK1-ICP* with the amino terminal DNA-binding domain in the *pGBKT7-Dest* vector as well as the empty vector as control were co-transformed into the *Saccharomyces cerevisiae* strain pJ69-4A together with the fusion constructs of the amino-terminal activation domain (AD) with *AHK1-ICP* (A; B; C), *IAA16* (A; B; C), *WAK4* (A; B; C), (A; B; *AT1G04780*), (A; B; *AT1G80350*), (A; B; *AT1G72250*), (A; B; *AT5G20470*), *MKKK20* (C), *MPK2* (C), *MPK3* (C), *MPK4* (C), *MPK5* (C), *MPK6* (C), *MPK7* (C), *MPK11* (C), *MPK17* (C). *pGADT7-Dest* was used as control. For the interaction test the respectively transformed yeast was plated on the auxotrophy medium CSM-Leu$^-$-Trp$^-$-Ade$^-$ (CSM-L-T-A) as well as on the growth control medium CSM-Leu$^-$-Trp$^-$ (CSM-L-T) and grown for four days at 28°C. The detection of expressed protein is shown in appendix A23.

## A27: Root elongation of *ahk1-3* and Ws-2 upon treatment with $CaCl_2$, flg22, different hormones and inhibitors of hormone signal transduction or hormone biosynthesis



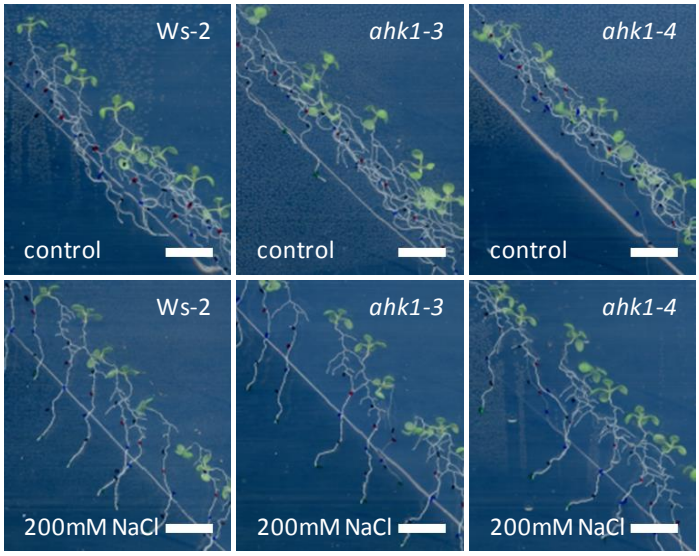

The diagrams show the root elongation in four days of *ahk1-3* (light grey, A) and its wildtype (dark grey, A) after the transfer of four day old seedlings to different concentrations of calcium chloride ($CaCl_2$, B), different concentrations of the pathogen-associated molecular pattern flg22 (C) as well as to different hormones and respective inhibitors (D). Analysed was the root elongation upon treatment with indole-3-acetic acid (IAA), the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA), methyl-jasmonate (MeJA), salicylic acid (SA), abscissic acid (ABA), giberellic acid 3 (GA3), 1-aminocyclopropane-1-carboxylic acid (ACC), the inhibitor of ethylene signal transduction silver nitrate (AgNO3) and kinetin (CK). The root elongation was measured using ImageJ. Shown are mean values and standard deviation of one experiment with at least 20 (B, C) or ten (D) seedlings per line and treatment. Student's t-test was used to calculate statistical significance. Stars above the bars display statistical significance in comparison to the respective wildtype: *p<0.05; ***p<0.001. Brackets around stars display that the significance has been shown in just one experiment.

## A28: Localization of RFP-IAA16 without transient co-expression with AHK1-GFP



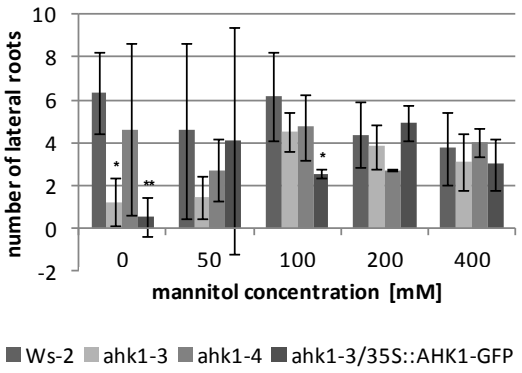

Transient expression of *IAA16* under the control of the *CaMV 35S-promoter* and tagged with a N-terminal *RFP* (*RFP-IAA16*, red) in *N. benthamiana* reveals in confokal microscopy that RFP-IAA16 localizes to the nucleus in general and during osmotic stress applied with 0.8M mannitol. The scale is 50µm.

## A29: Skewing of Ws-2, *ahk1-3* and *ahk1-4* in the halotropism assay performed by Dorota Kawa




Ws-2 (left), *ahk1-3* (middle) and *ahk1-4* (right) were exposed to a diagonal gradient of NaCl (bottom panel) and a control (upper panel). Pictures were taken five days after establishing the gradient. The scale is 1cm.

## A30: Number of lateral roots upon mannitol treatment




Mean values and standard deviations of lateral root number in twelve day old seedlings after eight days growth on mannitol-supplemented media. At least 19 seedlings per line and treatment were analyzed. Data were averaged between three replicates. Student‘s t-test was used to determine statistical significance. *p<0.05; **p<0.01.

## A31: Phosphoproteomic study of short-term kinetin treatment of Col-0 and *ahk2 ahk3*

Please cite this article in press as: Dautel et al., The Sensor Histidine Kinases AHK2 and AHK3 Proceed into Multiple Serine/Threonine/Tyrosine Phosphorylation Pathways in *Arabidopsis thaliana*, Molecular Plant (2015), http://dx.doi.org/10.1016/j.molp.2015.10.002

**Molecular Plant**
Letter to the Editor

CellPress
PARTNER JOURNAL

# The Sensor Histidine Kinases AHK2 and AHK3 Proceed into Multiple Serine/Threonine/Tyrosine Phosphorylation Pathways in *Arabidopsis thaliana*

Dear Editor,

Cytokinins are $N^6$-substituted adenine derivatives that are involved in the regulation of numerous aspects of plant growth and development. These include the control of cell division, leaf senescence, apical dominance, sink/source relationship, vascular and embryonic development, and apical meristem activity (Kieber and Schaller, 2014). In addition, there is increasing evidence for the function of cytokinins in abiotic stress responses (Zwack and Rashotte, 2015).

The cytokinin signal transduction pathway in plants is considered to be a canonical two-component, multistep phosphorelay system (TCS). Upon cytokinin binding, the sensor histidine kinases (HK) autophosphorylate at a conserved His residue. The phosphoryl group is then transferred to a conserved Asp residue in the HK's own receiver domain. The His-containing phosphotransfer proteins (HP) perceive the phosphoryl group from the receiver domain of the HKs and transfer it to the Asp in the receiver of response regulators (RR). The RRs function preferentially either as transcription factors mediating cytokinin-regulated gene expression (B-type RRs) or as negative regulators of cytokinin signaling (A-type RRs).

The cytokinin sensor HK family of *Arabidopsis* consists of three members: AHK2, AHK3, and AHK4. They have overlapping functions in cytokinin responses, are localized as dimers in the ER membrane, and bind cytokinin via their CHASE domain (Kieber and Schaller, 2014). To date, there is no evidence that AHK2, -3, and -4 and the TCS elements acting downstream of the HKs, namely the *Arabidopsis* HPs and RRs (ARR), have properties not related to a canonical His-to-Asp phosphorelay.

Interestingly, the osmolarity sensing pathway in yeast has been shown to be a canonical TCS phosphorelay, which eventually activates the Hog1 mitogen-activated protein kinase (MAPK) pathway upon high-osmolarity stress (Posas and Saito, 1998). Having this signaling transition from a TCS to a classical MAPK pathway in mind, we sought to test whether similar mechanisms may also exist in plants regarding cytokinin signaling.

To study the potential of the HK-dependent TCS phosphorelay to cause Ser/Thr/Tyr modification, comparative phosphoproteomics experiments in the absence or presence of the cytokinin were carried out with seedlings of the wild type (WT; Col-0) and the *ahk2ahk3* double mutant, which displays a relatively weak cytokinin hyposensitive phenotype (Higuchi et al., 2004). Seedlings were grown hydroponically for a reciprocal metabolic $^{15}$N-labeling approach under continuous white light for 14 days in media without cytokinin. Then the seedlings were either exposed to the final concentration of 100 ng/ml kinetin, a functional cytokinin, for 10 min or mock-treated before harvesting. The experiments were carried out in four biological replicates for each combination of $^{14}$N/$^{15}$N labeling, and the data were averaged between the replicates. We chose this early time point to identify phosphorylation/dephosphorylation events before the onset of major cytokinin-controlled gene expression and protein accumulation. Protein extraction, tryptic digestion, and phosphopeptide enrichment were carried out according to the Supplemental Information. Tryptic peptides were analyzed by liquid chromatography–tandem mass spectrometry using a Quadrupole-Orbitrap hybrid mass spectrometer. Proteins were identified by tandem mass spectrometry using information-dependent acquisition of fragmentation spectra of multiple charged peptides. Acquired spectra were matched against the *Arabidopsis* proteome. For quantitation, ratios between heavy ($^{15}$N) and light ($^{14}$N) forms of each peptide were calculated. Ratios from reciprocal experiments were converted to mutant-versus-WT ratios. Proteins significantly up- or downregulated in their phosphorylation pattern were defined by pairwise *t*-testing, if peptides were identified in both replica experiments. For peptides identified only in one replica experiment, a two-fold-change cutoff was applied.

In total, 836 phosphopeptides were identified in at least one genotype or condition (Supplemental Tables 1 and 2). Of these, 553 phosphopeptides upon kinetin treatment and 358 phosphopeptides under mock conditions were quantified and are presented as *ahk2ahk3*/WT ratio. In mock-treated seedlings, only very few significant differences in the phosphoproteome of the WT and *ahk2ahk3* seedlings were detectable (34 differentially phosphorylated proteins; Figure 1A and Supplemental Tables 1 and 2). In contrast, 150 phosphopeptides were found to be differentially modified between the WT and *ahk2ahk3* seedlings after kinetin treatment. There was an overlap of 12 phosphopeptides between mock- and kinetin-treated samples (Supplemental Table 3; the representative spectra for one of the overlapping phosphopeptides, LIEEVSHSSG(pS)PNPVSD, are exemplarily provided in Supplemental Figure 1). The differences of phosphorylation level in the *ahk2ahk3* double mutant compared with the WT were much more pronounced when plants were treated with kinetin (Supplemental Figure 2). Thus, there are major changes in the Ser/Thr/Tyr phosphoproteome that are dependent on the presence of AHK2 and AHK3, and these changes become particularly apparent in the presence of kinetin (Supplemental

Published by the Molecular Plant Shanghai Editorial Office in association with Cell Press, an imprint of Elsevier Inc., on behalf of CSPB and IPPE, SIBS, CAS.

Please cite this article in press as: Dautel et al., The Sensor Histidine Kinases AHK2 and AHK3 Proceed into Multiple Serine/Threonine/Tyrosine Phosphorylation Pathways in *Arabidopsis thaliana*, Molecular Plant (2015), http://dx.doi.org/10.1016/j.molp.2015.10.002



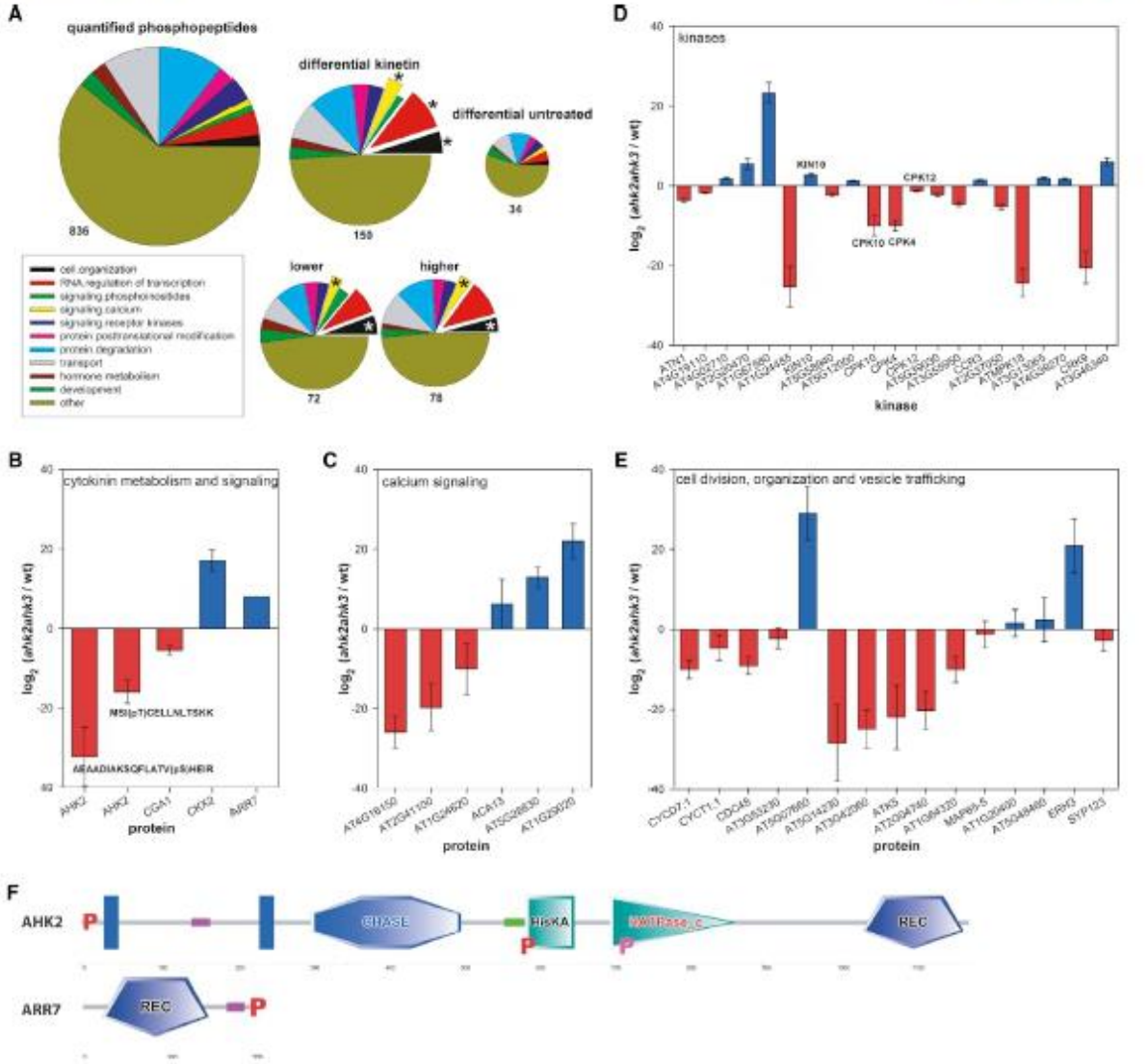



**Figure 1. The AHK2/AHK3-Dependent Two-Component Signaling System Causes Ser/Thr/Tyr Phosphorylation and Dephosphorylation in *Arabidopsis thaliana*.**

**(A)** Functional categorization of all identified phosphoproteins in the dataset ($n$ =836), the phosphoproteins differentially modified between the *ahk2ahk3* mutant and wild type (WT) after 10 min of kinetin (100 ng/ml) treatment ($n$ =150; kinetin dataset), with separation of higher ($n$ = 78) and lower ($n$ = 72) phosphorylation in the mutant, as well as the phosphoproteins differentially modified between the *ahk2ahk3* mutant and wt in the absence of kinetin ($n$ = 34). Diameters of the pie chart also represent the numbers of peptides included. The category "other" is composed of proteins with unknown function and proteins of the non-specified categories. Exploded pie slices marked with asterisks represent significantly over-represented functional categories ($p < 0.01$, Fisher exact test).

**(B–E)** Significant ($p < 0.05$, $t$-test) $\log_2$-fold phosphorylation changes of *ahk2ahk3* mutant versus WT within the kinetin dataset in proteins with function in cytokinin metabolism and signaling **(B)**, calcium signaling **(C)**, as protein kinases **(D)**, or in cell organization **(E)**. Blue bars indicate higher phosphorylation in the *ahk2ahk3* mutant; red bars indicate higher phosphorylation in WT. Error bars represent SD of two replica experiments, and multiple testing correction was applied.

**(F)** Schematic drawings of the domain structure of AHK2 and ARR7 according to the SMART database. Phosphorylation sites identified in this study are indicated by red letters P; pink letter P marks phosphorylation sites identified in other studies. Green bar indicates coiled-coil region and magenta bar indicates region of low complexity.

Figure 2). We therefore focused our further analysis on the dataset obtained from the kinetin-treated seedlings (hereafter called the kinetin dataset).

Of the 150 differentially modified phosphopeptides detected in the kinetin dataset, 72 displayed a reduced ($\log_2$ *ahk2ahk3*/wt $\leq -1$) and 78 phosphopeptides an increased ($\log_2$

Please cite this article in press as: Dautel et al., The Sensor Histidine Kinases AHK2 and AHK3 Proceed into Multiple Serine/Threonine/Tyrosine Phosphorylation Pathways in *Arabidopsis thaliana*, Molecular Plant (2015), http://dx.doi.org/10.1016/j.molp.2015.10.002



*ahk2ahk3*/wt ≥ 1) phosphorylation in the *ahk2ahk3* mutant compared with the WT. Given that almost equal numbers of proteins were found with increased and decreased phosphorylation, a large number of modification changes appear to be caused through changes in phosphatase activities. The 150 phosphopeptides contained 157 Ser, 59 Thr, and 23 Tyr sites. Using Motif-X and the standard settings of occurrence of a least 10 and a significance threshold of $10^{-6}$, no significant phosphorylation motifs were found, not even for the 157 Ser sites. For the Thr and Tyr sites the dataset is too small to allow efficient motif identification, even by using a lower significance threshold.

The differentially phosphorylated proteins have predicted or experimentally determined localization to the plasma membrane, ER, cytosol, nucleus, or extracellular space. Their functional categorization using Mapman demonstrates that the AHK2/AHK3-dependent TCS phosphorelay influences the modification state of many proteins (Figure 1A), particularly calcium signaling, cellular organization and division, and regulation of transcription (Figure 1A).

Interestingly, among the phosphopeptides differentially modified between *ahk2ahk3* double mutant and WT in the kinetin data set of WT seedlings we found AHK2 itself, which was phosphorylated at Thr4, a site previously identified in two other phosphoproteomics studies (Sugiyama et al., 2008; Nakagami et al., 2010), and at Ser596, very close to the His kinase domain of AHK2 (Figure 1B and 1F; Supplemental Figure 3). Both previous studies identified another AHK2 phosphorylation site at Thr740 located within the HATPase domain (Figure 1F). The AHK2 phosphorylation sites identified here were found in phosphorylated form in the kinetin-treated WT but not in the mock-treated WT datasets. ARR7, a canonical A-type ARR, shows increased phosphorylation within a predicted phosphorylation hotspot (Christian et al., 2012) in the *ahk2ahk3* mutant in the kinetin dataset at two adjacent sites (Ser197 and Ser199) in the C terminus (Figure 1B and 1F). ARR7 is a negative regulator of cytokinin signaling, and functions together with ARR15 in the cytokinin-controlled maintenance of the stem cell systems embedded in shoot and root meristems (Zhao et al., 2010). It is, therefore, tempting to speculate that the phosphorylation of AHK2 and dephosphorylation of ARR7 modulate the sensitivity state of the TCS phosphorelay, thereby influencing stem cell maintenance and also other cytokinin-related processes. Another protein with increased phosphorylation in *ahk2ahk3* in the kinetin dataset is cytokinin oxidase/dehydrogenase 2 (CKX2) (Figure 1B), which is predicted to be located in the ER lumen. CKX2 is a member of a multiprotein family, which catalyzes the breakdown of cytokinins. CKX2 phosphorylation may modulate its enzymatic activity and, thereby, the amount of active cytokinin in the ER lumen. Consequently, this might desensitize cytokinin perception as the cytokinin receptors expose their hormone-binding domain to the ER lumen (Schaller et al., 2015).

Among the phosphoproteins with differential phosphorylation between the *ahk2ahk3* mutant and WT in the kinetin dataset, we found an over-representation of the category "calcium signaling" (Figure 1A). Beside two calmodulin-like proteins (CML12: AT2G41100; CML25: AT1G24620) and two calcium-binding EF hand family proteins (AT5G28830, AT1G29020) of unknown physiological function, we identified one calmodulin-binding transcriptional regulator-like protein (AT4G16150) related to the *Oryza sativa* CaM-binding transcription factor (Choi et al., 2005), one calcium-transporting ATPase of the E1–E2 type (ACA13), probably exporting calcium ions out of the cell (Iwano et al., 2014), and three calcium-dependent protein kinases (CPKs) (Figure 1C and 1D). Although the functional role of the phosphorylation/dephosphorylation of these proteins has yet to be elucidated, the AHK2/AHK3-dependent TCS appears to influence calcium signaling and homeostasis.

We also identified 21 protein kinases among the AHK2/AHK3-dependent, differentially modified phosphoproteins in the kinetin data set (Figure 1D). One of these was KIN10, with an enhanced phosphorylation in the mutant at Thr6 and Tyr19, N-terminal to the kinase domain. KIN10 is one of the two main catalytic subunits of the *Arabidopsis* SNF1-related kinase 1 (SnRK1). Upon sensing energy stress, SnRK1 triggers both transcriptional and non-transcriptional changes that contribute to metabolic reprogramming and restoration of energy homeostasis (Baena-González and Sheen, 2008). Assuming that the phosphorylations trigger the activity of SnRK1, a link is established to how the AHK2/AHK3-dependent TCS could promote long-term effects in plant cell survival, differentiation, and development at the level of metabolic and energy homeostasis. Another three prominent kinases, which showed a reduced phosphorylation in the mutant compared with the WT in the kinetin dataset, are the three CPKs mentioned above, namely CPK4, CPK10, and CPK12 (Figure 1D and Supplemental Table 1). These CPKs function in abscisic acid, drought, and salinity signaling in a calcium-dependent manner (Boudsocq and Sheen, 2013). Whether these modifications influence the activity of the CPKs is not yet known; however, one may hypothesize that the functional interaction of the AHK2/AHK3-dependent TCS signaling and abiotic stress responses (Zwack and Rashotte, 2015) might be the result of modification of CPKs. We also found the *Arabidopsis* MAP kinase 18 (ATMPK18) to have reduced phosphorylation in the mutant compared with the WT in the kinetin dataset (Figure 1D and Supplemental Table 1). The phosphorylations occur at Ser415 and Ser426, C-terminal to the kinase domain. Phosphorylation of S426 was also found in a phosphoproteomics study of DNA-repair mechanism (Roitinger et al., 2015). Furthermore, ATMPK18 forms a signaling module with the dual-specificity MAPK phosphatase, PROPYZAMIDE HYPERSENSITIVE 1, which regulates cortical microtubule functions (Walia et al., 2009). Consistently, a significant number of phosphoproteins with functions in "cell organization" were identified in the kinetin dataset, which is differentially modified in an AHK2/AHK3-dependent manner (Figure 1A). These proteins are mainly cytoskeletal components or are involved in processes such as cell division, cell organization, and vesicle trafficking (Figure 1E). With respect to cell division, we identified one cyclin-dependent protein kinase (CYCD7;1), one cyclin (CYCT1;1), two cell division cycle proteins (CDC48, AT3G53230), and a component of the SMC5/6 complex (SMC6A: AT5G07660) (Figure 1E). Regarding cell organization, three different myosin heavy chain-related proteins (AT3G42060, AT1G20400, AT1G64320), two ankyrin repeat-containing proteins (AT5G14230, AT2G04740), one putative actin-binding (AT5G48460) and one microtubuli-associated (MAP65-5) protein, as well as the kinesin-5 motor protein (ATK5)

Please cite this article in press as: Dautel et al., The Sensor Histidine Kinases AHK2 and AHK3 Proceed into Multiple Serine/Threonine/Tyrosine Phosphorylation Pathways in *Arabidopsis thaliana*, Molecular Plant (2015), http://dx.doi.org/10.1016/j.molp.2015.10.002



were found (Figure 1E). Finally, the katanin-like protein ERH3 and one syntaxin (SYP123) showed AHK2/AHK3-dependent differential modification. These results are in agreement with the function of the AHK2/AHK3-dependent TCS in the regulation of cell division and cell growth (Kieber and Schaller, 2014), and may add a new level of complexity to the control of these cellular processes.

The AHK2/AHK3-dependent changes in the Ser/Thr/Tyr phosphoproteome of *Arabidopsis* raise the question of how the AHK cytokinin receptors or their downstream TCS elements achieve this. From a thermodynamic point of view canonical HKs, HPs, and RRs are not able to phosphorylate Ser, Thr, or Tyr directly. Therefore, other mechanisms must exist that enable the AHK2/AHK3-dependent TCS elements to activate canonical Ser/Thr/Tyr kinases or phosphatases, which may then be responsible for the majority of protein modification found in our phosphoproteome data set. At least two possible and complementary mechanisms are conceivable, which have the physical interaction of the TCS elements with putative target kinases in common. In the first case an interaction with TCS elements may induce autophosphorylation followed by autoactivation of the target kinases. These kinases may cause an initial wave of phosphorylation. An example of this mechanism is the autophosphorylation and activation of the MAPKKKs Ssk2 by physical interaction with the canonical response regulator Ssk1 in the yeast Hog1 pathway (Posas and Saito, 1998). In the second case the association of TCS elements with kinases results in their activation without autophosphorylation. Such kinases are then responsible for the modification of secondary kinases (and phosphatases), which in turn phosphorylate downstream targets. An *in planta* example of this mechanism is the conformational regulation of MAPKKK-like constitutive triple response 1 (CTR1) by interaction with the ethylene receptors, members of the HK family (Wang et al., 2013). Both mechanisms could take place in the ER membrane, at the ER–cytosol interface, in the cytosol, or in the nucleus, as the TCS elements of the AHK2/AHK3 signaling pathway are found in all these compartments (Kieber and Schaller, 2014). Interestingly, although the cytokinin receptor-like family of monocots consists for the most part of canonical HKs, *O. sativa* AtCRE1-like 4 (OsCRL4) is predicted to be a Ser/Thr kinase (Han et al., 2004). This opens the possibility that at least in rice, *Os*CRL4 might be able to cause rapid changes in Ser/Thr phosphorylation in a direct manner.

Our study revealed a previously unrecognized massive signaling transition from the AHK2/AHK3-dependent TCS phosphorelay to Ser/Thr/Tyr phosphorylation/dephosphorylation. In future, it will be worth exploring (1) how and at which molecular level within the TCS (HK, HP, or RR) and where in the cell this transition occurs, (2) what the consequences of the modification are for the functional properties of the identified proteins, and (3) how the entire phosphorelay–phosphorylation network is organized.

## SUPPLEMENTAL INFORMATION

Supplemental Information is available at *Molecular Plant Online*.

## FUNDING

This work was supported by a grant from the DFG to K.H. (SFB 1101, project B05) and by a fellowship from the Landesgraduiertenförderungsprogramm of Baden-Württemberg, Germany, to R.D.

## AUTHOR CONTRIBUTIONS

R.D., X.N.W., and M.H. conducted the experiments; W.X.S. and K.H. designed the experiments and wrote the paper.

## ACKNOWLEDGMENTS

No conflict of interest declared.

Received: June 12, 2015
Revised: September 3, 2015
Accepted: October 9, 2015
Published: October 17, 2015

*Rebecca Dautel*[1], *Xu Na Wu*[2], *Michael Heunemann*[1], *Waltraud X. Schulze*[2] *and Klaus Harter*[1,*]

[1]Department of Plant Physiology, Center for Plant Molecular Biology (ZMBP), University of Tübingen, Auf der Morgenstelle 32, 72076 Tübingen, Germany
[2]Department of Plant Systems Biology, University of Hohenheim, 70593 Stuttgart, Germany
*Correspondence: Klaus Harter (klaus.harter@zmbp.uni-tuebingen.de)
http://dx.doi.org/10.1016/j.molp.2015.10.002

## REFERENCES

**Baena-González, E., and Sheen, J.** (2008). Convergent energy and stress signalling. Trends Plant Sci. **13**:474–482.

**Boudsocq, M., and Sheen, J.** (2013). CDPKs in immune and stress signalling. Trends Plant Sci. **18**:30–40.

**Choi, M.S., Kim, M.C., Yoo, J.H., Moon, B.C., Koo, S.C., Park, B.O., Lee, J.H., Koo, Y.D., Han, H.J., Lee, S.Y., et al.** (2005). Isolation of a calmodulin-binding transcription factor from rice (*Oryza sativa* L.). J. Biol. Chem. **280**:40820–40831.

**Christian, J.O., Braginets, R., Schulze, W.X., and Walther, D.** (2012). Characterization and prediction of protein phosphorylation hotspots in *Arabidopsis thaliana*. Front. Plant Sci. **3**:207.

**Han, Q.-M., Jiang, H.-W., Qi, X.-P., Yu, J., and Wu, P.** (2004). A CHASE domain containing protein kinase OsCRL4, represents a new AtCRE1-like gene family in rice. J. Zhejiang Univ. Sci. **5**:629–633.

**Higuchi, M., Pischke, M.S., Mähönen, A.P., Miyawaki, K., Hashimoto, Y., Seki, M., Kobayashi, M., Shinozaki, K., Kato, T., Tabata, S., et al.** (2004). In planta functions of the *Arabidopsis* cytokinin receptor family. Proc. Natl. Acad. Sci. USA **101**:8821–8826.

**Iwano, M., Igarashi, M., Tarutani, Y., Kaothien-Nakayama, P., Nakayama, H., Moriyama, H., Yakabe, R., Entani, T., Shimosato-Asano, H., Ueki, M., et al.** (2014). A pollen coat-inducible autoinhibited $Ca^{2+}$-ATPase expressed in stigmatic papilla cells is required for compatible pollination in the Brassicaceae. Plant Cell **26**:636–649.

**Kieber, J.J., and Schaller, G.E.** (2014). Cytokinins. *Arabidopsis* Book **12**:e0168.

**Nakagami, H., Sugiyama, N., Mochida, K., Daudi, A., Yoshida, Y., Toyoda, T., Tomita, M., Ishihama, Y., and Shirasu, K.** (2010). Large-scale comparative phosphoproteomics identifies conserved phosphorylation sites in plants. Plant Physiol. **153**:1161–1174.

**Posas, F., and Saito, H.** (1998). Activation of the yeast SSK2 MAP kinase kinase kinase by the SSK1 two-component response regulator. EMBO J. **17**:1385–1394.

**Roitinger, E., Hofer, M., Köcher, T., Pichler, P., Novatchkova, M., Yang, J., Schlögelhofer, P., and Mechtler, K.** (2015). Quantitative phosphoproteomics of the ataxia telangiectasia-mutated (ATM) and ataxia telangiectasia-mutated and rad3-related (ATR) dependent DNA damage response in *Arabidopsis thaliana*. Mol. Cell. Proteomics **14**:556–571.

Please cite this article in press as: Dautel et al., The Sensor Histidine Kinases AHK2 and AHK3 Proceed into Multiple Serine/Threonine/Tyrosine Phosphorylation Pathways in *Arabidopsis thaliana*, Molecular Plant (2015), http://dx.doi.org/10.1016/j.molp.2015.10.002



**Schaller, G.E., Bishopp, A., and Kieber, J.J.** (2015). The Yin-Yang of hormones: cytokinin and auxin interactions in plant development. Plant Cell **27**:44–63.

**Sugiyama, N., Nakagami, H., Mochida, K., Daudi, A., Tomita, M., Shirasu, K., and Ishihama, Y.** (2008). Large-scale phosphorylation mapping reveals the extent of tyrosine phosphorylation in *Arabidopsis*. Mol. Syst. Biol. **4**:e1–7.

**Walia, A., Lee, J.S., Wasteneys, G., and Ellis, B.** (2009). *Arabidopsis* mitogen-activated protein kinase MPK18 mediates cortical microtubule functions in plant cells. Plant J. **59**:565–575.

**Wang, F., Cui, X., Sun, Y., and Dong, C.H.** (2013). Ethylene signaling and regulation in plant growth and stress responses. Plant Cell Rep. **32**:1099–1109.

**Zhao, Z., Andersen, S.U., Ljung, K., Dolezal, K., Miotk, A., Schultheiss, S.J., and Lohmann, J.U.** (2010). Hormonal control of the shoot stem-cell niche. Nature **465**:1089–1092.

**Zwack, P.J., and Rashotte, A.M.** (2015). Interactions between cytokinin signaling and abiotic stress responses. J. Exp. Bot. **66**:4863–4871.

## SUPPLEMENTAL INFORMATION

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Plant Material

For this study the *Arabidopsis thaliana* T-DNA insertional mutant *ahk2-2ahk3-3* (Higuchi et al., 2004) and the Columbia-0 (Col-0) wild type (wt) were used.

### Seedling growth and experimental design

20mg *Arabidopsis thaliana* seeds of wt and *ahk2ahk3* were germinated and grown under continuous light (22°C, 40µmol$m^{-2}s^{-1}$) in 50 ml JPL medium containing 30µM $H_3BO_3$, 30µM $MnSO_4$, 11.1µM $ZnSO_4$, 1.5µM KJ, 0.3µM $Na_2MoO_4$, 30nM $CoCl_2$, 30nM $CuSO_4$, 4mM $CaCl_2$, 1mM $MgSO_4$, 5µM $FeSO_4$, 5µM $Na_2EDTA$, 37.5µM $KH_2PO_4$, 10µM $NaH_2PO_4/Na_2HPO_4$, 0.5% sucrose, 1mM Glutamine, 3mM MES pH5.8, 2mM $KNO_3$, 1mM $NH_4NO_3$, shaking with 80u/min. After 10 days the JPL medium was renewed. After 14 days the media in which the seedlings were growing were supplemented with Kinetin to a final concentration 100 ng/ml. After 10 min shaking at 80 u/min in kinetin-supplemented media the seedlings were harvested, frozen in liquid nitrogen and stored at -80°C. In a control experiment (mock treatment) the seedlings were not treated with kinetin and harvested directly.

We used $^{15}N$-labeling and a reciprocal experimental design (Kierszniowska et al., 2009). These experiments were carried out in four biological replicates for each combination of $^{14}N/^{15}N$-labeling. Data were averaged between these replicates.

### Protein extraction, tryptic digestion and phophopeptide enrichment

Protein extraction, tryptic digestion and phosphopeptide enrichment was carried out as described in Wu and Schulze (2015).

**Mass spectrometric data analysis and statistics**

Tryptic peptide mixtures were analyzed by LC/MS/MS using nanoflow Easy-nLC1000 (Thermo Scientific) as an HPLC-system and an Quadrupole-Orbitrap hybrid mass spectrometer (Q-Exactive Plus, Thermo Scientific) as a mass analyzer. Peptides were eluted from a 75µm x 50cm analytical column (Thermo Scientific) on a linear gradient running from 4 to 64% acetonitrile in 120min and sprayed directly into the LTQ-Orbitrap mass spectrometer. Proteins were identified by MS/MS using information-dependent acquisition of fragmentation spectra of multiple charged peptides. Up to twelve data-dependent MS/MS spectra were acquired for each full-scan spectrum acquired at 60,000 full-width half-maximum resolution. Overall cycle time was approximately one second (Wu et al., 2014). Acquired spectra were matched against the *Arabidopsis* proteome (TAIR10, 35386 entries) using Mascot v.2.2. Thereby, carbamidomethylation of cysteine was set as a fixed modification; oxidation of methionine as well as phosphorylation of serine, threonine and tyrosine was set as variable modifications. Mass tolerance for the database search was set to 20ppm on full scans and 0.5Da for fragment ions and " $^{15}$N-metabolic labeling" was chosen as quantitation option. Peptides were accepted using a FDR threshold of 0.01. For quantitation, ratios between heavy ($^{15}$N) and light forms of each peptide were calculated using Mascot Distiller. Hits to contaminants (e.g. keratins) and additionally identified reverse hits were excluded from further analysis. Ratios from reciprocal experiments were converted to "mutant vs wild type" ratios and averaged. Significantly up- or down-regulated proteins were defined by pairwise t-testing with multiple-testing correction (Benjamini et al, 1995) if peptides were identified in both replica experiments. For the peptides identified only in one replica experiment, a two-fold-change cutoff was applied. A full list of identified and regulated peptides is in supplementary tables 1 and 2.

SUPPLEMENTAL REFERENCES

**Benjamini, Y., Hochberg, Y.** (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Royal Stat. Soc. **57**: 289-300.

**Brenner, W.G., Romanov, G.A., Köllmer, I., Bürkle, L., Schmülling, T.** (2005). Immediate-early and delayed cytokinin response genes of Arabidopsis thaliana identified by genome-wide expression profiling reveal novel cytokinin-sensitive processes and suggest cytokinin action through transcriptional cascades. Plant J. **44**: 314-333.

**Kierszniowka, S., Seiwert, B., Schulze, W.X.** (2009). Definition of Arabidopsis sterol-rich membrane microdomains by different treatment with methyl-β-cyclodextrin and quantitative proteomics. Mol. Cell. Proteomics **8**: 612-623.

**Mok, D.W.S., Mok, M.C.** (2001). Cytokinin metabolism and action. Annu. Rv. Plant Physiol. Plant Mol. Biol. **52**: 89-118.

**Schultz, J., Milpertz, F., Bork, P., Ponting, C.P.** (1998). SMART, a simple modular architecture research tool: Identification of signaling domains. Proc. Natl. Acad. Sci. USA **95**:5857-5864.

**Schwartz, D., Gygi, S.P.** (2005). An iterative statistical approach to the identification of protein phosphorylation motifs from large-scale data sets. Nat. Biotech. **23**: 1391-1398.

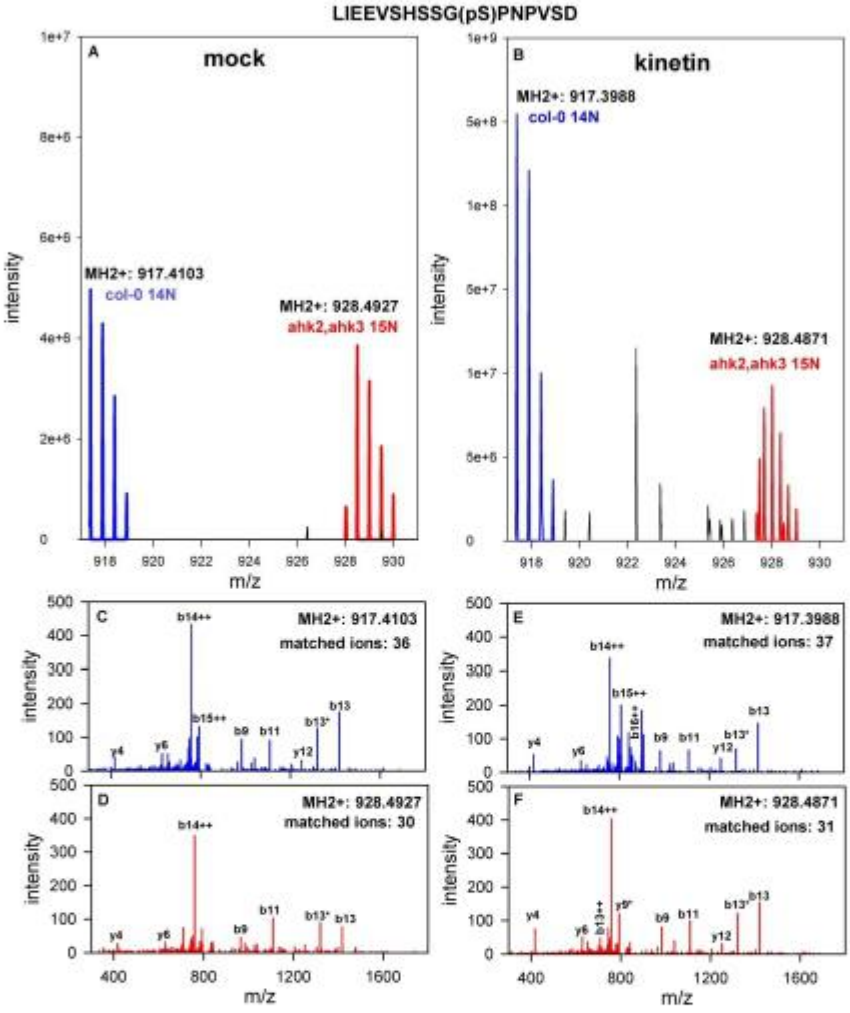
**Tanz, S.K., Castleden, I., Hooper, C.M., Vacher, M., Small, I., Millar, H.A.** (2013). Suba3: a database for integrating experimentation and prediction to define the subcellular location of proteins in Arabidopsis. Nucleic Acids Res. **41**: D1185-91.

**Thimm, O., Bläsing, O., Gibon, Y., Nagel, A., Meyer, S., Kruger, P., Selbig, J., Müller, L.A., Seung, Y.R., Stitt, M.** (2004). Mapman: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J. **37**: 914-939.

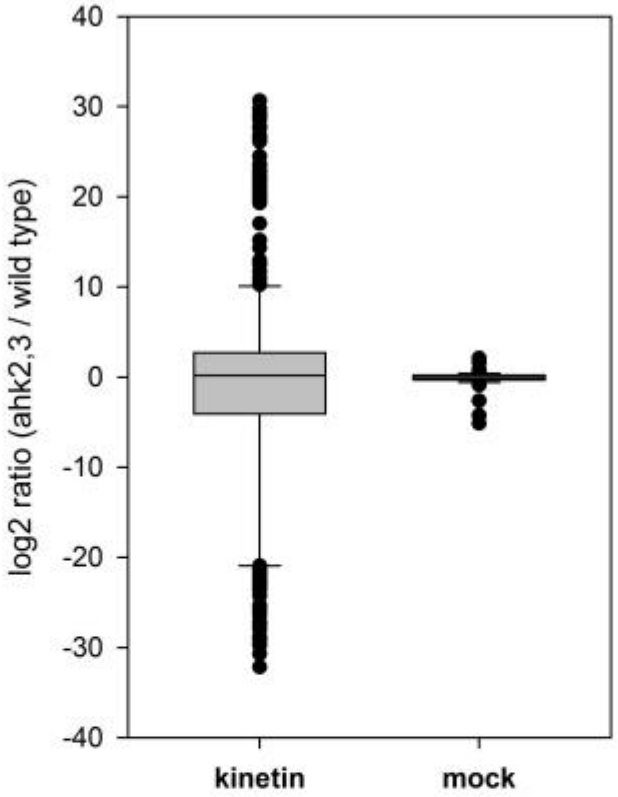
**Van Wijk, K.J., Friso, G., Walther, D., Schulze, W.X.** (2014). Meta-analysis of Arabidopsis thaliana phospho-proteomics data reveals compartmentalization of phosphorylation motifs. Plant Cell **26**: 2367-2389.

**Wu, X.N., Schulze, W.X.** (2015). Phosphopeptide profiling of receptor kinase mutants. Meth. Mol. Biol. **1306**: 71-79.

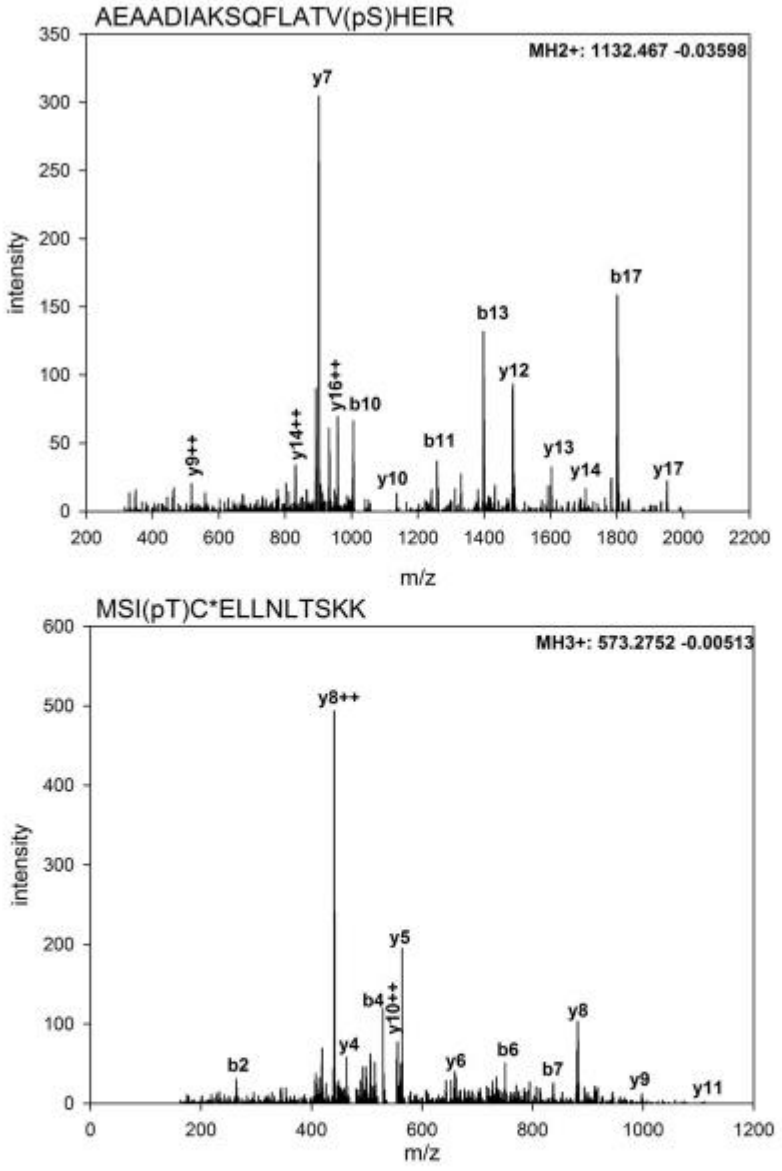
**Wu, X.N., Sklodowski, K., Encke, B., Schulze, W.X.** (2014). A kinase-phosphatase signaling module with Bsk8 and Bsl2 involved in regulation of sucrose-phosphate synthase. J. Proteome Res. **13**: 3397-3409.




**SUPPLEMENTAL FIGURE 1.** Representative spectra of the phosphopeptide LIEEVSHSSG(pS)PNPVSD. Full scan spectra of the $^{14}N$ (wild type) and $^{15}N$ (ahk2ahk3 double mutant) labeled peptides under mock (A) and kinetin treatment (B). In this example, four independent fragment spectra were obtained for the unlabeled (C) and labeled (D) peptide under mock treatment, and for the unlabeled (E) and labeled (F) peptide under kinetin treatment. Labels within the fragment spectra indicate prominent signature ions; the respective total number of ions matching the peptide sequence is noted besides the parent ion mass.




**SUPPLEMENTAL FIGURE 2**. Distribution of phosphopeptide $log_2$ ratios between the *ahk2ahk3* mutant and wild type in the kinetin treated and mock treated experiments. Note, that upon kinetin treatment, the differences in the phosphopeptide distribution between the *ahk2ahk3* mutant and wild type are much stronger compared to mock treatment.




**SUPPLEMENTAL FIGURE 3.** Spectra of Ser/Thr phosphorylation sites MSI(pT)CELLNLTSKK Thr4 and AEAADIAKSQFLATV(pS)HEIR Ser596 identified upon kinetin application. Matching ions are labelled according to the peptide fragmentation nomenclature (Roepstorff and Fohlman, 1984). C* indicates carbamindomethylation of cysteine.

(**Roepstorff, P., Fohlman, J.** (1984). Proposal for a common nomenclature for sequence ions in mass spectra peptides. Biomed. Mass Spectrom. **11**: 601)

The additional supplemental data of this publication can be found on the attached CD.

Supplemental table 1: ahk2 ahk3 regulated phosphopeptides and ORA

Supplemental table 2: ahk2 ahk3 quantified phosphopeptides

Supplemental table 3: overlap of phosphopeptides between mock- and kinetin-treatment

## A32: My contribution to the publication Dautel *et al.* (2016)

My contribution to the publiclation Dautel *et al.* (2016) with the title “The Sensor Histidine Kinases AHK2 and AHK3 Proceed into Multiple Serine/Threonine/Tyrosine Phosphorylation Pathways in *Arabidopsis thaliana*.” was to produce the growth media and to cultivate the *ahk2 ahk3* and Col-0 seedlings to obtain the samples for protein extraction, phosphoprotein enrichment and phosphoprotein quantification. Furthermore I participated in the generation of the figures.

## A33: Phosphopeptides which were quantified in *ahk1*-3 and Ws-2 in an experiment with a reciprocal metabolic labeling experimental design and 10min treatment with 0.3M mannitol

Phosphopeptides (pPeptides) which were quantified in *ahk1-3* (ahk1) and the wildtype (wt) Ws-2 in an experiment with metabolic labeling after 10min treatment with 0.3M mannitol, are listed in the tables of their respective functional category. The $log_2$-values of the ratio of normalized phosphopeptide ion intensities of *ahk1-3* and Ws-2 ($log_2$(ahk1/wt)) reveal more pPeptide abundance in *ahk1-3* ($log_2$>0) or in wt ($log_2$<0). sd gives the standard deviation, p-values show the respective statistical significance. Data were obtained from Waltraud X. Schulze.

cell.organisation

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G04780 | (ac)M(ox)ASS(ph)TIDVTK | 10.00 | | | 10.00 | 0.3299 | |
| AT1G08730 | RS(s)(s)ATLFGRMSQSFR | 4.21 | 0.5500 | | 2.91 | | |
| AT1G08730 | DGLAKT(ph)VYSR | -43.13 | | | | | |
| AT1G14500 | M(ox)FVT(ph)FVK | 2.12 | | | | | |
| AT1G18370 | LLDDPEKGT(ph)VVEK | 2.32 | | | | | |
| AT1G18550 | LESLNAS(ph)VS(ph)R | 3.88 | | | | | |
| AT1G20060 | T(ph)FTKVTTTTK | -20.21 | | | | | |
| AT1G24150 | VLAQS(ph)EGDNK | 29.50 | | | | | |
| AT1G29170 | NELPSM(ox)VT(ph)S(ph)APKPEIK | 28.99 | | | | | |
| AT1G42550 | TSFSVPSPKMT(ph)S(ph)R | -23.68 | 0.0932 | | | | |
| AT1G72250 | T(ph)LQDKVKELESQLLVER | 2.43 | 0.1200 | | 1.93 | 0.8203 | |
| AT1G77550 | AVFEAAALAHPEM(ox)QS(ph)PK | | | | | | |
| AT1G80260 | S(ph)RVNSFGIDCLESLK | 26.44 | | | | | |
| AT1G80350 | GGATSKS(ph)TAGAR | -41.61 | | | | | |
| AT2G22610 | DEQS(ph)QEAVLLRQKIK | -20.38 | | | | | |
| AT2G34730 | KTEEKLS(ph)ETK | -21.13 | | | | | |
| AT2G37080 | S(ph)EM(ox)ETM(ox)QSEKNK | 7.14 | 0.2980 | | | | |
| AT2G37420 | TAMIS(ph)DASSNIR | -19.88 | | | | | |
| AT3G44730 | ELEEVKS(ph)NFVETR | 11.95 | | | | | |
| AT4G03830 | AAS(ph)VDLLPISK | 5.54 | | | 4.32 | 0.0727 | |
| AT4G05190 | ENIES(ph)LQEKLS(ph)KEK | -21.50 | | | | | |
| AT4G16340 | EKLSEDFY(ph)FQIQPTEM(ox)QDAK | 31.01 | 0.0313 | | | | |
| AT4G19660 | EAFLHFLS(ph)Y(ph)IY(ph)T(ph)GR | | | | 0.28 | | |
| AT4G25590 | M(ox)KMVYAS(ph)SK | -19.65 | | | | | |
| AT4G27370 | FRNAIS(ph)LESKT(ph)IEK | -21.83 | | | | | |
| AT4G34380 | SSSSPHEGS(ph)GAWSSRNVEK | -21.28 | | | | | |
| AT4G39050 | MLAGEIAFS(t)(s)TLK | 29.04 | | | | | |
| AT5G10470 | Y(ph)LGVLAEKSR | -20.36 | 0.0783 | | | | |
| AT5G12320 | RLILAGASLS(ph)LLNR | 0.88 | | | | | |
| AT5G20470 | FNEY(ph)LNM(ox)S(ph)LK | 2.07 | | | 1.83 | | |
| AT5G41310 | ATSMS(ph)LK | 2.44 | | | | | |
| AT5G48460 | MNATY(ph)VISIAR | -9.71 | | | | | |

cell.vesicle transport

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G04750 | IELLVDKT(ph)ENLR | -22.04 | | | | | |
| AT1G56610 | VFS(ph)QATLVTLK | -0.73 | | | | | |
| AT2G28640 | AHNLVT(ph)IAM(ox)K | 28.40 | | | | | |
| AT2G28650 | RS(ph)VGM(ox)M(ox)IIPR | 0.25 | | | | | |
| AT3G08790 | FKKPT(ph)SGRAGSM | -2.30 | | | | | |
| AT3G11820 | (ac)M(ox)NDLFS(ph)SSFSRFR | | | | 1.95 | | |
| AT3G29400 | S(ph)GKSLTKNAK | -1.37 | 0.8000 | | -3.99 | | 5.99E-02 |
| AT3G49420 | (ac)M(ox)M(ox)S(ph)FEMNDR | | | | -0.76 | | |
| AT3G55480 | ASAGLLRIGTDAHLYDDPEDVNIAPLLDS(ph)KFESEK | -22.62 | | | | | |
| AT4G23460 | M(ox)ERGTFLET(ph)WK | -22.12 | 0.1661 | | | | |
| AT5G03540 | (ac)AVDS(ph)RM(ox)DLLSER | -0.13 | 0.4326 | 8.69E-02 | -1.37 | 0.3808 | 8.69E-02 |
| AT5G16880 | LKIGGS(ph)EVSNK | 11.16 | | | | | |
| AT5G50440 | M(ox)LEDSFQSGVAILS(ph)K | -0.59 | | | 0.18 | 0.2595 | 2.96E-01 |

development.

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G30610 | PS(ph)PVTYGLIM(ox)EVM(ox)LACEK | -10.00 | | | -10.00 | | |
| AT1G32400 | 109.94 AANTPAEYDS(ph)DDEYLAPR b10/y9 . | -21.59 | 0.2287 | | | | |
| AT1G55540 | PPMPQSNS(ph)PFTISPIS(ph)AS(ph)K | -10.00 | | | -10.00 | 0.8452 | |
| AT1G72410 | RSSIVSDM(ox)S(ph)T(ph)DLASEKK | | | | | | |
| AT2G18060 | PSS(ph)S(ph)M(ox)S(ph)IT(ph)SMDNNYNYK | | | | -0.33 | 0.3163 | |
| AT2G19430 | T(ph)GKCVKVIGSQDK | 0.94 | | | | | |
| AT2G32950 | M(ox)EEI(s)(t)DPVVPAVKPDPR | 4.18 | | | | | |
| AT2G41980 | NLALY(ph)FS(ph)GSDKEELK | 2.98 | 0.4500 | | -0.97 | | |
| AT2G42200 | RRKPQPAS(ph)LS(ph)VLASR | 2.02 | | | | | |
| AT3G05680 | LAVGT(ph)LMGPQK | -20.96 | | | | | |
| AT3G09090 | IT(ph)QSQIY(ph)DRPGK | -24.31 | | | 0.08 | | |
| AT3G12850 | NDLESAT(ph)DELEK | -0.80 | | | | | |
| AT3G19430 | VVPIT(ph)M(ox)EDSRIHGY(ph)DVK | | | | -10.00 | | |
| AT3G61190 | M(ox)M(ox)T(ph)KTLEIDLR | -20.73 | | | | | |
| AT4G00060 | KPTPEAKSDKNVNLS(ph)T(ph)K | 0.14 | | | | | |
| AT4G01450 | LPTNT(ph)VEEEK | 0.93 | 0.0600 | | 1.35 | | |

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT4G13560 | AADLT(ph)QS(ph)ARDKT(ph)ADGSHSANK | -3.24 | | | | | |
| AT4G26370 | PT(ph)NLVS(ph)LRT(ph)GNK | 10.00 | | | 10.00 | | |
| AT5G12130 | FIPVT(ph)SSYDGNR | -20.50 | | | | | |
| AT5G14120 | REDQEPGLQT(ph)PDLILS(ph)EVEDEKPK | | | | | | |
| AT5G16780 | T(ph)DEFGRT(ph)LTPK | -0.05 | | | | | |
| AT5G59460 | REISS(ph)TLR | -20.29 | | | | | |
| AT5G60910 | NFMGEDLDS(ph)LSLK | 1.26 | 0.2010 | 2.16E-01 | -0.18 | 0.6382 | |

## protein.degradation

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G02980 | QAT(ph)EAATDKAASTSGLK | -20.45 | | | | | |
| AT1G04730 | EPHVRQS(ph)ESSDIKGCK | -27.21 | | | | | |
| AT1G07200 | M(ox)EDLT(ph)ASVT(ph)NR | -21.20 | | | 0.99 | | |
| AT1G11750 | (ac)M(ox)AGLAISPPLGLSFS(ph)S(ph)RTR | | | | -0.28 | 1.1657 | |
| AT1G16470 | (ac)M(ox)GDSQYSFSLTTFS(ph)PS(ph)GK | | | | 0.79 | 0.3415 | |
| AT1G19460 | LGLS(ph)ET(ph)FLY(ph)AAIR | | | | -1.42 | | |
| AT1G23260 | (ac)M(ox)S(ph)S(ph)EEAKVVVPR | | | | -10.00 | | |
| AT1G45000 | LIVKAS(ph)SGPR | 10.23 | | | | | |
| AT1G47350 | SART(ph)ENGEILSLARK | -21.62 | | | | | |
| AT1G49630 | GLKLLSAAS(ph)R | 28.51 | | | | | |
| AT1G50980 | ILS(ph)LLPS(ph)KDVVAT(ph)GVLS(ph)K | | | | | | |
| AT1G51320 | QYKM(ox)LDY(ph)YK | 60.57 | | | | | |
| AT1G65040 | M(ox)ILAT(ph)T(ph)T(ph)VSIIVK | | | | 10.00 | | |
| AT1G65110 | NIS(ph)DDIVLKSIDLLK | 28.19 | | | | | |
| AT1G70960 | (ac)M(ox)VNTS(ph)FETLPR | | | | 2.75 | | |
| AT1G74370 | EGFISKGEEAAT(ph)KPRR | 2.31 | | | -2.90 | | |
| AT1G75400 | QVS(ph)DSQILGLK | 0.37 | | | | | |
| AT1G77650 | WTTRGLDRY(ph)NK | -44.88 | | | | | |
| AT2G07240 | RPRTLS(ph)SKLDGR | 4.03 | | | 0.60 | | |
| AT2G18190 | VM(ox)NS(ph)YLSHVVAESEETK | -0.68 | | | | | |
| AT2G18915 | EFT(ph)THEATAWRK | -23.22 | | | | | |
| AT2G21500 | S(ph)PKEIHSPSSLR | -20.57 | | | | | |
| AT2G24280 | NIS(s)(s)IVALVT(ph)KK | -19.44 | | | | | |
| AT2G24540 | LPM(ox)VLAKY(ph)DSAVIGK | -0.25 | | | 0.90 | | |
| AT2G29860 | EPVLYAFIGCT(ph)PYTTPRWFILR | -2.48 | | | | | |
| AT2G39720 | ENFVLKS(ph)SAR | 10.00 | | | 10.00 | | |
| AT2G42730 | NLM(ox)MLPRAS(ph)SKY(ph)R | -22.54 | | | | | |
| AT2G43260 | KLS(ph)PPPYVVNVGSK | -21.73 | | | | | |
| AT2G44130 | FPDT(ph)SPR | 1.53 | | | | | |
| AT2G45920 | Y(ph)SM(ox)RM(ox)ADLLSTK | 0.26 | | | | | |
| AT3G12775 | EKEGVS(ph)FFVR | 0.31 | | | | | |
| AT3G16550 | (ac)VSRYSRALLPT(ph)IT(ph)IS(ph)SR | | | | -0.63 | | |
| AT3G17270 | S(ph)LNT(ph)MY(ph)AKLK | 10.00 | | | 10.00 | | |
| AT3G22700 | S(ph)ARTWY(ph)TLSER | -0.97 | | | | | |
| AT3G27110 | RS(ph)VS(ph)YIGFGAEKVGR | -20.83 | | | | | |
| AT3G27330 | S(ph)T(ph)IS(ph)FST(ph)AVTAR | | | | -10.00 | | |
| AT3G28510 | NYLAS(ph)KSTALAKR | -3.51 | | | | | |
| AT3G28580 | LKANTTKGS(ph)K | 29.33 | | | | | |
| AT3G28600 | LTFHRRS(ph)R | -20.76 | | | | | |
| AT3G42550 | DT(ph)S(ph)ILLSALY(ph)YTTVQIGTPPR | -20.47 | | | | | |
| AT3G49150 | RM(ox)VIKQCS(ph)FVNT | -19.48 | | | | | |
| AT3G53970 | LVT(ph)DLQS(ph)EIIDK | -23.23 | | | | | |
| AT3G60820 | M(ox)S(ph)TGYSILSR | 0.20 | | | | | |
| AT4G02760 | SLKLGSISSSAEPTTS(ph)LLT(ph)R | 30.12 | | | | | |
| AT4G05475 | M(ox)ATSTT(ph)LQSLLMK | -21.31 | | | | | |
| AT4G09920 | NYDRHPY(ph)MIENMPK | -1.41 | | | | | |
| AT4G17510 | AT(ph)ASESSSSKR | 10.48 | | | | | |
| AT4G17740 | FSYARS(ph)RSNISR | | | | | | |
| AT4G20310 | T(ph)SNGS(ph)LYLGGS(ph)RR | 10.00 | | | 10.00 | | |
| AT4G22060 | QIFET(ph)QVNYDILM(ox)EK | 7.55 | | | | | |
| AT4G23580 | SLLASTELYQT(ph)R | 5.04 | | | | | |
| AT4G26350 | WGS(ph)LWRWVPK | -1.17 | | | | | |
| AT4G36550 | HS(ph)IIILKNLCS(ph)TEKGR | -23.51 | | | | | |
| AT5G02310 | IENM(ox)INQS(ph)LTR | -21.47 | | | | | |
| AT5G02880 | VLDLPLS(ph)K | 2.64 | | | | | |
| AT5G05740 | GNLRGKPATSY(ph)EK | 29.49 | | | | | |
| AT5G06460 | ENIIAS(ph)AS(ph)SPM(ox)KK | -0.01 | 0.0500 | | 0.10 | | |
| AT5G17760 | IS(ph)KGHKDK | -21.63 | | | | | |
| AT5G22010 | ST(s)(s)KAGPVKNAAETAPIK | -22.39 | | | | | |
| AT5G22660 | Y(ph)FLENSLVLK | 1.51 | | | | | |
| AT5G22980 | MFY(ph)FFFESRNK | 0.20 | | | | | |
| AT5G38396 | NVQCLDLS(ph)ANTLEMLSLSCES(ph)MPVFK | -21.92 | | | | | |
| AT5G41490 | (ac)MAT(ph)MITNLR | | | | -1.81 | | |
| AT5G43060 | VVTIDSYEDVPENSEASLK | -21.63 | | | | | |
| AT5G43060 | VVT(ph)IDSYEDVPENSEASLKK | -11.30 | | | | | |
| AT5G45390 | MGTLSLSSSLKPS(ph)LVS(ph)S(ph)R | -40.76 | | | | | |
| AT5G46210 | LRAGNKGT(ph)S(ph)EEELESVLEK | 10.56 | | | | | |
| AT5G46740 | RFT(ph)NDGVTMEK | -22.37 | | | | | |
| AT5G56430 | LIPT(ph)LDY(ph)DGTY(ph)S(ph)AAALEFFGK | | | | 2.32 | | |
| AT5G56560 | LSS(ph)VKLS(ph)VASLLK | 5.33 | | | | | |
| AT5G57820 | AKDDEKTLNM(ox)DS(ph)K | -3.02 | | | 1.39 | | |

## protein.posttranslational modification

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G03590 | LLS(ph)LLNSIKSK | 1.06 | | | | | |
| AT1G43900 | SSLM(ox)ISSRDPNALFS(ph)GGGIS(ph)FLAGVR | -21.32 | | | | | |
| AT1G61610 | TGDLVLCS(ph)DSDR | 0.42 | | | | | |
| AT1G66700 | DKM(ox)T(ph)KAIS(ph)ANLDLDLISNR | -0.27 | 0.0185 | | | | |
| AT1G71530 | S(ph)ILQLLPHHPPS(ph)S(ph)SSSSK | -13.66 | | | -10.00 | | |
| AT2G23080 | AAE(s)(s)RLRTQ | -23.56 | | | | | |
| AT2G25880 | (ac)M(ox)GIS(ph)TETQQIAASEAAQKR | 1.33 | | | -0.93 | 0.6411 | 5.48E-02 |
| AT2G30020 | LRFQKPPS(ph)GFAPGPLS(ph)FGS(ph)ES(ph)VSASSPPGGVLK | 29.66 | | | 6.99 | | |
| AT3G50310 | DEDKVLMS(ph)PK | 2.84 | | | | | |
| AT3G51370 | SIGDVY(ph)LKK | 28.46 | | | | | |
| AT3G53640 | RSYS(ph)PSDEVVK | 28.93 | | | | | |

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT3G53930 | NVTNLTAPMPIASAT(ph)GNNLSR | -22.37 | | | | | |
| AT3G59110 | NSGKMMSY(ph)LGRTK | 1.57 | | | | | |
| AT4G02710 | S(ph)QT(ph)IVLLNESIK | -4.19 | 0.2133 | | -3.90 | | 1.07E-01 |
| AT4G24100 | EM(ox)VAMCLVKDQT(ph)K | -0.21 | 0.2998 | | | | |
| AT5G21222 | QKHFHSLLS(ph)LISK | -23.26 | | | | | |
| AT5G25510 | TAVLVT(ph)PR | -40.84 | | | | | |
| AT5G26751 | DST(ph)GVDKLPEEM(ox)NDMK | 0.20 | 0.0200 | | -0.57 | | |
| AT5G35380 | FKAADV(s)(s)TVM(ox)K | 0.34 | | | | | |
| AT5G35960 | (ac)M(ox)LKCVS(ph)VQIQTSFVLS(ph)CLLLLQSIAMK | | | | 10.00 | | |
| AT5G39420 | VS(ph)DLPM(ox)T(ph)TGPASGFAWAVK | -2.18 | 1.3900 | | -3.90 | | |
| AT5G53140 | QT(ph)DVAFLESEK | 0.53 | | | | | |
| AT5G57610 | IFLFS(ph)T(ph)PEQDGSLHY(ph)VER | -2.68 | 0.1094 | | | | |
| AT5G57670 | Y(ph)DDQQM(ox)NK | 0.13 | | 3.11E-01 | -0.11 | | |
| AT5G59270 | DTLMDVVDS(ph)KLGDFKAK | 6.96 | | | | | |
| AT5G63370 | M(ox)KEDRFEEEYGFPLT(ph)S(ph)LR | -19.33 | | | | | |
| AT5G65530 | LT(ph)RHAKEVEER | 1.69 | | | | | |

## RNA.regulation of transcription

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G01060 | DTNT(ph)SGEELLAK | 0.75 | | | | | |
| AT1G20900 | RPRGRPPGS(ph)KNK | -19.58 | | | | | |
| AT1G21000 | GDLSLTFS(ph)LK | 1.65 | | | 0.14 | | |
| AT1G22985 | KQDS(ph)DASGGASEEVV | 0.11 | | | | | |
| AT1G26680 | S(ph)Y(ph)FVGS(ph)VTASSIKKDK | -5.60 | | | | | |
| AT1G28420 | AELS(ph)EKLDLSDR | -21.90 | | | | | |
| AT1G34410 | LFGVT(ph)LDTPPM(ox)IK | 4.23 | | | 3.12 | | |
| AT1G49190 | S(ph)DRLDQVK | -20.44 | | | | | |
| AT1G49900 | RNQDEVVPS(ph)RDK | -20.82 | | | -0.37 | | |
| AT1G58025 | VKLKT(ph)S(ph)K | 0.79 | | | | | |
| AT1G59890 | DRNS(ph)TFPGMHPK | -23.01 | | | | | |
| AT1G61980 | ETLLDKSS(ph)KSEK | 0.31 | | | | | |
| AT1G62110 | YS(ph)VILHGRR | 10.69 | | | | | |
| AT1G63470 | S(ph)KDS(ph)SSM(ox)SDPNAPK | -11.47 | | | | | |
| AT1G72440 | FVTALDES(ph)SK | 2.20 | | | | | |
| AT1G73360 | FAS(ph)LSVPASSSR | 1.22 | | | | | |
| AT1G77800 | ESLLKM(ox)AVS(ph)GPPS(ph)EKR | | | | -2.03 | | |
| AT1G79430 | GLT(ph)LYHLKSHLQK | -21.60 | | | | | |
| AT1G79700 | Y(ph)LNPNAAADK | -0.28 | | | | | |
| AT2G03710 | ARSM(ox)LDQLS(ph)DLKTK | -43.95 | | | -0.80 | | |
| AT2G20710 | FFET(ph)IPM(ox)ERR | | | | 10.00 | | |
| AT2G23340 | (ac)MET(ph)EAAVTATVTAATM(ox)GIGT(ph)R | | | | -0.37 | | |
| AT2G23380 | TTPTKF(s)(s)K | 1.30 | | | | | |
| AT2G26780 | Y(ph)PKFIEMLEY(ph)ILK | 2.18 | 0.1300 | | -0.10 | | |
| AT2G26780 | T(ph)DTEEDSRTTTRETITGK | 0.46 | | | | | |
| AT2G28090 | MDKEKETVT(ph)VM(ox)GT(ph)MDIK | -20.28 | | | | | |
| AT2G28500 | TDAVNS(ph)M(ox)VYEAGAR | 0.60 | | | | | |
| AT2G28700 | KFEM(ox)LPET(ph)QK | 0.04 | | | | | |
| AT2G36720 | LSDS(ph)S(ph)LGIIQTKQER | 1.43 | | | | | |
| AT2G38950 | GPGYTLKSFKNFADT(ph)Y(ph)K | 0.51 | | | | | |
| AT2G44730 | Y(ph)TASPSAGVSSNPR | -21.37 | 0.3879 | | | | |
| AT2G45660 | IENATSRQVT(ph)FSK | 4.63 | | | | | |
| AT2G46020 | VAST(ph)S(ph)KLHVSS(ph)PKS(ph)GR | 0.40 | 0.1737 | | -0.14 | 0.8169 | |
| AT3G04730 | TYQDLSNALS(ph)K | -21.57 | | | -8.31 | | |
| AT3G05380 | S(ph)SETTHK | -10.00 | | | -10.00 | | |
| AT3G10800 | TFNGNTNKPT(ph)SSSSMVVSVLLDPR | 29.52 | | | | | |
| AT3G18960 | MVTTQNT(ph)KAR | 10.00 | | | 10.00 | | |
| AT3G21480 | KLS(ph)PEEERGFS(ph)PGGVVTR | 12.51 | | | | | |
| AT3G21810 | FDERRDY(ph)AGGLK | -0.85 | 0.1210 | | | | |
| AT3G24050 | KKT(ph)M(ox)T(ph)VAAAALIM(ox)GR | -22.40 | | | | | |
| AT3G24140 | SLM(ox)PGSY(ph)VQR | 1.31 | | | | | |
| AT3G24490 | VEKS(ph)GLGSSK | 2.07 | 0.3517 | | | | |
| AT3G27720 | KCEDES(ph)ETVNWMTVNTK | 11.34 | | | | | |
| AT3G46080 | S(ph)FLPETTTVTTLK | 1.72 | | | | | |
| AT3G47500 | S(ph)PEKVT(ph)PELS(ph)DK | 1.01 | | | -0.43 | 1.1176 | 3.49E-01 |
| AT3G48050 | LWWLT(ph)DQDYIDDRQLEVDK | -0.72 | | | | | |
| AT3G52250 | EEDILPIPS(ph)MK | -0.69 | 0.1000 | | 0.37 | 0.0612 | |
| AT3G52270 | Y(ph)MGKNRQIQVIDNAR | 0.27 | | | | | |
| AT3G53460 | MS(ph)ASASSLSAFNPK | 0.07 | | | | | |
| AT3G55080 | M(ox)LFCIS(ph)TVK | -2.19 | | | | | |
| AT3G56330 | SEVQIERNLEFETGET(ph)FFRHES(ph)AR | -20.27 | | | | | |
| AT4G08990 | T(ph)FQLTM(ox)ASLLEIGY(ph)QVR | 28.67 | | | | | |
| AT4G11060 | IRMIKY(ph)GES(ph)ISK | 0.71 | | | | | |
| AT4G11140 | SPVS(ph)VLESPFSGESTAVK | -19.20 | | | | | |
| AT4G12040 | LCDNGCGFFGS(ph)PS(ph)NM(ox)NLCSK | -1.28 | | | | | |
| AT4G13980 | TTLKSQELNFNS(ph)IE(t)(s)ASEK | -21.26 | | | | | |
| AT4G13980 | LLNFLET(ph)AIR | 6.33 | | | | | |
| AT4G22745 | KSRSENSSVASSGS(ph)K | 1.78 | | | | | |
| AT4G31610 | KT(ph)PSPFLIVK | -20.50 | | | | | |
| AT4G31800 | QSPEIEQT(ph)DIPIKK | 2.89 | | | | | |
| AT4G38000 | IKT(ph)T(ph)AKPPR | 60.31 | | | | | |
| AT4G39870 | (ac)M(ox)GKHKS(ph)FR | 0.63 | | | 0.39 | 0.4095 | 5.82E-01 |
| AT5G04340 | SFAT(ph)GQALGGHK | 0.00 | | | | | |
| AT5G06550 | M(ox)PKCKNLLL(t)(s)K | -20.79 | | | | | |
| AT5G07350 | IGIWQYGDIESDDEDTGPAR | -0.95 | 0.2209 | | | | |
| AT5G09790 | M(ox)KS(ph)MAEIM(ox)AK | -10.00 | | | -10.00 | | |
| AT5G11430 | T(ph)ALKDEAAKADNEK | 3.20 | | | | | |
| AT5G16680 | RQRS(ph)S(ph)LLAGAK | -22.38 | | | | | |
| AT5G18560 | HT(ph)LPQTVLPAASFK | -0.09 | | | | | |
| AT5G19490 | IAM(ox)AVPLLVS(ph)K | -1.50 | | | | | |
| AT5G22260 | LKT(ph)FGESGHPAEM(ox)NELSFR | -2.56 | | | | | |
| AT5G22650 | KKGGHT(ph)AT(ph)PHPAK | 0.04 | 0.3028 | | 0.09 | | |
| AT5G23150 | S(ph)AS(ph)VGERLTVVSK | -21.27 | | | | | |
| AT5G23150 | TTSPVS(ph)ESLEHSSFDPKIK | 29.42 | | | | | |
| AT5G36670 | SEVKVES(ph)KDDR | -20.84 | | | -3.89 | | |
| AT5G51230 | M(ox)PGIPLVS(ph)R | 5.03 | | | | | |
| AT5G52170 | KLT(ph)LESKQIK | -20.27 | | | | | |
| AT5G58850 | T(ph)KISIE(t)R(s)DNK | -24.55 | | | | | |

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT5G59800 | FRS(ph)LVSVER | -4.74 | | | | | |
| AT5G60170 | IDSSINT(ph)DKK | 5.04 | | | | | |
| AT5G60200 | KPS(ph)PAT(ph)AVTR | | | | 10.00 | | |
| AT5G65210 | ALSSSWAT(ph)RHREPT | 3.24 | | | | | |

## signaling.

(Ca) calcium; (G) G-proteins; (S) sugar and nutrients; (L) light; (M) MAP kinases; (PI) phoshpoinositides; (RK) receptor kinases; (U) unspecified

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G04830 | (G) | S(ph)RITDEDHPLSLGK | 10.60 | | | | | |
| AT1G07150 | (M) | M(ox)LS(ph)S(ph)PSSFWVR | | | 2.36E-01 | -1.21 | | 1.99E-01 |
| AT1G09630 | (G) | AFQTILS(ph)EVY(ph)RIIS(ph)KK | | | | -10.00 | | |
| AT1G09630 | (G) | ARRPDEEY(ph)DYLFK | -0.69 | | | | | |
| AT1G10240 | (L) | MCQS(ph)IKEKDPNFK | 4.64 | | | | | |
| AT1G14390 | (RK) | NS(ph)TQNLAQQMEVLSKLR | 29.91 | | | 3.90 | | |
| AT1G18840 | (Ca) | LQGS(ph)SSPRQLGTT(ph)EK | -0.52 | 0.5668 | | | | |
| AT1G19090 | (RK) | LLGFLRAM(ox)S(ph)SVNDFITNDK | 10.99 | | | | | |
| AT1G21210 | (RK) | HIVSYFASAT(ph)K | 0.99 | | | 2.39 | 0.1804 | 1.07E-01 |
| AT1G21230 | (RK) | IMGEERPS(ph)M(ox)K | 2.76 | 0.1000 | 8.84E-02 | 3.91 | 0.1911 | 4.52E-02 |
| AT1G23540 | (RK) | MVQVVRALDCDGDS(ph)GDISNGIK | -22.42 | | | | | |
| AT1G29020 | (Ca) | DKAKIT(ph)SDTFSEIYK | 11.16 | | | | | |
| AT1G51820 | (RK) | YLFRGY(ph)ISNSSTRIR | -44.44 | | | | | |
| AT1G53430 | (RK) | RLGPIPEY(ph)IGS(ph)MS(ph)ELK | -2.95 | 0.0867 | | | | |
| AT1G53510 | (M) | FS(ph)KADPLALR | -1.66 | | | | | |
| AT1G56330 | (G) | Y(ph)HLGLT(ph)NFT(ph)TGK | -2.91 | 3.3600 | | -3.98 | | 6.69E-02 |
| AT1G64460 | (PI) | T(ph)LRIST(ph)M(ox)LLKK | -0.87 | 0.2140 | 4.16E-01 | -0.03 | 0.6050 | 4.17E-01 |
| AT2G03150 | (G) | GGKDES(ph)RIQVK | -21.10 | 0.2600 | | | | |
| AT2G17930 | (PI) | M(ox)LDAGKS(ph)LCSLLK | -22.01 | | | | | |
| AT2G19190 | (RK) | GQIDPAFS(ph)NLT(ph)SIRKLDLSGNTLTGEIPAFLANLPNLTELNVEGNK | 12.97 | | | | | |
| AT2G26420 | (PI) | FDLKGS(ph)SHGRTIDK | 0.30 | 0.0700 | | -1.99 | | |
| AT2G28960 | (RK) | MGIT(ph)PENASLPLR | -14.56 | | | | | |
| AT2G39280 | (G) | PSLQAIEDLMS(ph)VR | | | | 0.20 | | |
| AT2G40116 | (PI) | ETLKVKVY(ph)MGDGWR | -23.62 | | | | | |
| AT2G41560 | (Ca) | M(ox)VT(ph)GDNISTAK | 11.04 | | | | | |
| AT2G43130 | (G) | (ac)SDDDERGEEYLFK | | | | 10.00 | | |
| AT3G02880 | (RK) | LIEEVSHSSGSPNPVSD | | | | -1.57 | 0.1083 | 1.56E-02 |
| AT3G05990 | (RK) | ELS(ph)VTPAGAVIFASR | -0.31 | 0.0500 | 3.84E-01 | 0.09 | | |
| AT3G13460 | (Ca) | QVS(ph)EEKVT(ph)DEKK | 28.91 | | | | | |
| AT3G19320 | (RK) | S(ph)FFYLPCGKDPHR | 4.76 | | | | | |
| AT3G21180 | (Ca) | M(ox)STSS(ph)S(ph)NGLLLTSM(ox)SGR | | | | -10.00 | 0.0809 | |
| AT3G45780 | (L) | (ac)M(ox)EPTEKPSTKPS(ph)SR | 1.69 | | | 0.03 | 0.4140 | 3.16E-01 |
| AT3G46270 | (RK) | TKYY(ph)YENYDDK | 10.00 | | | 10.00 | | |
| AT3G50840 | (L) | VY(ph)DDGLY(ph)RAVDIYFK | 10.89 | | | | | |
| AT3G51830 | (PI) | VSTIYGVGGT(ph)IR | -22.67 | | | | | |
| AT3G55660 | (G) | T(ph)PTKIDDFGFKR | -20.12 | | | | | |
| AT3G57830 | (RK) | Y(ph)VHGNLKSTK | -20.22 | | | | | |
| AT3G57830 | (RK) | KL(s)(s)T(ph)VSTPEK | 28.76 | | | | | |
| AT3G63150 | (G) | (m)(m)LGGKS(ph)SAGGR | 1.42 | | | | | |
| AT4G11110 | (L) | ARNMDQQT(ph)VAS(ph)SGSALVIANTSAK | 0.50 | | | | | |
| AT4G14750 | (Ca) | M(ox)QRSSSQLGS(ph)NTAK | 1.01 | | | | | |
| AT4G17160 | (G) | Y(ph)IIIGDTGVGK | 1.29 | | | | | |
| AT4G23270 | (RK) | KIDLNAS(ph)QSLYGMVR | -2.58 | | | -3.91 | | |
| AT4G23280 | (RK) | PTM(ox)SAIVQM(ox)LT(ph)T(ph)SS(ph)IALAVPR | | | | 0.02 | | |
| AT4G33240 | (PI) | EY(ph)KQMLNVVK | 1.96 | | | 2.80 | | |
| AT4G35310 | (Ca) | EM(ox)FQAMDTDNSGAIT(ph)FDELKAGLR | -21.00 | 0.1092 | | | | |
| AT4G36945 | (PI) | SESSS(ph)LDTMSR | -21.69 | | | | | |
| AT4G38200 | (G) | S(ph)SSAEIRELIVR | 28.05 | | | | | |
| AT4G38430 | (G) | FPGLPQT(ph)TLDMNK | -22.51 | | | | | |
| AT5G05160 | (RK) | AVLEDT(ph)TAVVVK | 2.13 | 0.2016 | | | | |
| AT5G05940 | (G) | FPSLTQTS(ph)LDISK | 1.92 | | | | | |
| AT5G05940 | (G) | M(ox)ENLVKS(ph)CAGIEK | 37.18 | | | | | |
| AT5G06740 | (RK) | DIAVKRVS(ph)EK | 30.48 | | | | | |
| AT5G10530 | (RK) | GY(ph)LNSLDMMVAIK | 0.61 | | | | | |
| AT5G16900 | (RK) | RIT(ph)YSEILLM(ox)TNNFER | 25.34 | | | | | |
| AT5G17470 | (Ca) | QT(ph)IAECIAM(ox)VR | 1.22 | | | | | |
| AT5G17580 | (L) | LY(ph)EPFM(ox)IRAIKS(ph)R | -0.38 | | | | | |
| AT5G39030 | (RK) | ASIPES(ph)KSLIK | 4.37 | | | | | |
| AT5G39380 | (Ca) | SLDDNLNETLKPGS(ph)S(ph)KM(ox)KK | -22.27 | | | | | |
| AT5G39380 | (Ca) | GTVS(ph)SRVASKK | 4.76 | | | | | |
| AT5G43310 | (L) | ET(ph)RVSLDTQNKSVSQTR | 11.63 | 0.6031 | | 4.34 | | |
| AT5G43310 | (L) | LS(ph)EPKMGNT(ph)SAPSSSVRPRR | -1.86 | | | | | |
| AT5G46330 | (RK) | M(ox)NLT(ph)FISIGR | -19.63 | | | | | |
| AT5G48380 | (RK) | LK(t)F(s)V(s)DNRLVGPIPNFNQTLQFK | -47.46 | 0.2185 | 5.48E-02 | -14.91 | | |
| AT5G48410 | (S) | FNGLS(ph)GDFQLNDK | -22.91 | | | 3.19 | | |
| AT5G54590 | (RK) | CIS(ph)RAPR | -19.76 | | | -10.00 | | |

## stress.

(U) unspecified; (a) abiotic; (b) biotic

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G21610 | (a) | M(ox)EPS(ph)TTSNQQPKK | 7.30 | | | -3.89 | | |
| AT1G33870 | (b) | M(ox)T(ph)QLLER | -21.69 | | | | | |
| AT1G33880 | (b) | GTS(ph)VSKPVK | -3.45 | | | | | |
| AT1G58390 | (b) | MVCS(ph)GGGFPQLKK | 6.25 | | | | | |
| AT1G62320 | (a) | LM(ox)KS(ph)LIQGFLPGIVLK | -1.71 | | 3.16E-01 | -12.70 | | |
| AT1G63730 | (b) | M(ox)VET(ph)IARDVSNK | 0.62 | | | | | |
| AT1G68300 | (a) | NAGLNRLDEGT(ph)K | -21.04 | | | | | |
| AT1G77310 | (a) | KSGSNGRPKYS(ph)TLEK | -26.13 | | | | | |
| AT3G02840 | (b) | NQLIS(ph)GDISVVETK | -22.12 | | | | | |
| AT3G23010 | (b) | IM(ox)DTFPFWLGS(ph)LPY(ph)LK | 10.00 | | | 10.00 | | |
| AT3G44630 | (b) | LETLPT(ph)NINLIS(ph)LR | -19.92 | | | | | |
| AT4G02100 | (a) | S(ph)ESIAHVLSHIK | 28.88 | | | 10.00 | | |
| AT4G08685 | (a) | CS(ph)NVSPGHDRARVTLTR | 29.50 | | | | | |
| AT4G15910 | (a) | (ac)M(ox)AARS(ph)LSGAVK | 0.63 | | | 0.29 | 0.6327 | 5.74E-01 |
| AT4G16660 | (a) | IEKVT(ph)KT(ph)ENTTK | 0.21 | 0.0300 | | 0.01 | | |
| AT4G16860 | (b) | YDVFPS(ph)FSGVDVRK | -0.05 | | | | | |
| AT4G21100 | (a) | IFVLPDLT(ph)LIT(ph)K | | | | 10.00 | | |

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT4G25200 | (a) | AS(ph)ALALKRLLSSSIAPR | -14.39 | | | | | |
| AT4G29920 | (a) | RT(ph)GAYTVHQT(ph)LTPEAAS(ph)VLK | 10.00 | | | 10.00 | | |
| AT4G39640 | (a) | SELVAVS(ph)DPR | -2.41 | | | | | |
| AT5G09590 | (a) | REVVSSPFSAY(ph)R | 1.29 | 0.1400 | | 1.01 | | |
| AT5G11250 | (b) | RGES(ph)IGPELLR | -44.40 | | | | | |
| AT5G17890 | (b) | EIPIFNAT(ph)HPPK | -2.30 | | | | | |
| AT5G40170 | (b) | S(ph)LVNC(t)(t)LK | -21.10 | | | | | |
| AT5G45050 | (b) | YAGLQEIY(ph)K | 5.12 | | | | | |
| AT5G51440 | (a) | VY(ph)KTDEIKAEM(ox)K | 33.44 | | | | | |
| AT5G64940 | (a) | FDYEPIAAAS(ph)LGQVHR | 1.62 | | | | | |

## transport.

(ABC) ABC transorters; (aa) amino acids; (am) ammonium; (Ca) calcium; (cyn) cyclic nucleotide or calcium regulated channels; (H+) H+ transportig pyrophosphatase; (Aq) Major Intrinsic Proteins; (U) membrane system unknown; (met) metabolite transporters; (m) metall; (mi) misc; (ni) nitrate; (nu) nucleotides; (ATP) p- and v-ATPases; (pep) peptides; (por) porins; (px) peroxisomes; (P) phosphate; (K) potassium; (su) sugars; (S) sulphate; (-) anions; (+) cations

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G05030 | (su) | M(ox)WVTNT(ph)VLLY(ph)R | 0.87 | | 7.12E-02 | -0.36 | 0.0817 | 9.82E-02 |
| AT1G15990 | (cyn) | FIPLT(ph)SELK | 4.06 | 0.2100 | 7.18E-02 | | | |
| AT1G16780 | (H+) | GSDS(ph)HKAAVTGDTVGDPFK | -20.65 | | | | | |
| AT1G17500 | (mi) | YNLIT(ph)FFPK | -0.56 | | | -0.79 | | |
| AT1G22530 | (mi) | EILQSES(ph)FK | 0.50 | | 9.63E-01 | -0.19 | 1.0639 | |
| AT1G59870 | (ABC) | (ac)M(ox)DYNPNLPPLGGGGVS(ph)M(ox)RR | | | | 0.29 | 0.7550 | |
| AT1G59870 | (ABC) | GT(ph)ADFLQEVTSKK | -22.59 | 0.0312 | | | | |
| AT1G60960 | (m) | FPFPGFFAMIAALIT(ph)LFVDFM(ox)GTQYYERKQER | 10.00 | | | 10.00 | | |
| AT1G61630 | (+) | (ac)M(ox)TNPEDIPS(ph)R | 1.61 | | 5.13E-01 | -0.31 | 2.0615 | 6.46E-01 |
| AT1G67300 | (su) | LLYS(ph)M(ox)FSTFCLMAVMFVK | -21.85 | | 2.24E-02 | -5.98 | | 6.69E-02 |
| AT1G69870 | (pep) | RISS(ph)PGSILDAEK | | | | 3.88 | | |
| AT1G69870 | (pep) | S(ph)SPSELDVVDPYK | | | | 10.00 | | |
| AT1G71880 | (su) | KLYS(ph)LGVQS(ph)GAM(ox)GLMFNSIVLGFMSLGVEWIGRK | -14.70 | 0.0859 | | -1.94 | | |
| AT1G72160 | (mi) | SM(ox)IPQNLGS(ph)FK | -2.47 | 0.1615 | | -1.31 | 0.8303 | 2.52E-01 |
| AT1G75370 | (mi) | T(ph)KLM(ox)WS(ph)NM(ox)IK | -0.76 | | | | | |
| AT2G01980 | (+) | (ac)MTTVIDAT(ph)MAYR | 0.33 | 0.2040 | | 0.10 | | |
| AT2G18960 | (ATP) | GLDIDTAGHHYT(ph)V | 1.00 | 0.4008 | 1.57E-01 | 0.77 | 0.0778 | 4.33E-02 |
| AT2G22500 | (met) | NY(ph)KSVLDAITQM(ox)IR | -22.37 | | | | | |
| AT2G31910 | (m) | (ac)M(ox)ADPAAES(ph)IDASSS(ph)RFGR | | | | 1.14 | | |
| AT2G31910 | (m) | T(ph)TASLLIM(ox)NDEAKPK | | | | 10.00 | | |
| AT2G37170 | (Aq) | SLGS(ph)FRSAANV | -14.94 | 0.3513 | 2.95E-01 | -1.70 | 0.8329 | 1.80E-02 |
| AT2G39450 | (m) | IS(ph)NIANM(ox)LLFAAK | -0.80 | | | | | |
| AT2G40420 | (aa) | SSLAGES(ph)T(ph)TY(ph)AGVM(ox)K | | | | -0.79 | | |
| AT2G47800 | (ABC) | S(ph)FLGSHIVEDGSK | 1.65 | 0.1926 | | | | |
| AT3G01390 | (ATP) | (ac)M(ox)ES(ph)NRGQGSIQQLLAAEVEAQHIVNAAR | | | | 10.00 | | |
| AT3G09030 | (K) | RGMIS(ph)KIEAGGDR | -0.07 | | | | | |
| AT3G13090 | (ABC) | ALLVAMT(ph)GFK | -13.38 | | | | | |
| AT3G21250 | (ABC) | LRSTSEILNS(ph)MKVIK | 28.52 | 0.0522 | | | | |
| AT3G27870 | (ATP) | LLLLS(ph)KGADSVMFKR | -13.87 | | | | | |
| AT3G28380 | (ABC) | M(ox)IGKFS(ph)TALR | -19.63 | | | -1.01 | | |
| AT3G45680 | (pep) | EVKTSAAMPS(ph)KSFR | -21.22 | | | | | |
| AT3G47770 | (ABC) | (ac)M(ox)AKPVAAS(ph)FLT(ph)QANALFK | | | | 1.74 | 0.2495 | |
| AT3G49920 | (por) | TLDKY(ph)PR | -22.21 | | | | | |
| AT3G51670 | (mi) | (ac)M(ox)DASLS(ph)PFDHQKTQNTEPK | 0.19 | | | -0.35 | 1.2180 | 5.23E-01 |
| AT3G53420 | (Aq) | SLGS(ph)FRSAANV | -0.50 | 0.0679 | 7.64E-03 | -1.70 | 0.8329 | 5.02E-03 |
| AT3G60160 | (ABC) | EVY(ph)LAYLT(ph)T(ph)VK | | | | -10.00 | | |
| AT4G11440 | (met) | GSVKKSS(ph)IK | 3.81 | | | | | |
| AT4G13510 | (am) | VEPRS(ph)PSPSGANTTPTPV | | | | 0.81 | | |
| AT4G21120 | (aa) | M(ox)AS(ph)GGGDDGLRR | -20.06 | | | -4.31 | | |
| AT4G23700 | (m) | M(ox)AEHPGIS(ph)LTVVR | -1.99 | 0.1828 | | | | |
| AT4G30190 | (ATP) | GLDIETPSHYT(ph)V | -9.76 | 0.1710 | 1.85E-02 | -3.70 | | 1.17E-03 |
| AT4G32500 | (K) | RKLS(ph)NFK | -20.78 | | | | | |
| AT4G32530 | (ATP) | (ac)SGVVALGHASS(ph)WGAALVR | 10.00 | | | 10.00 | | |
| AT4G35100 | (Aq) | ALGS(ph)FRSNATN | -21.23 | 0.1060 | 1.42E-01 | -12.98 | 0.2479 | |
| AT5G06530 | (ABC) | FRDVTY(ph)KVVIK | 0.67 | | | | | |
| AT5G16740 | (aa) | S(ph)LPSTM(ox)TDRTKLVAR | 11.06 | | | | | |
| AT5G23630 | (+) | ELILT(ph)T(ph)FK | 2.28 | | | | | |
| AT5G35730 | (P) | FSRPSTISNLLY(ph)GR | -13.51 | | | | | |
| AT5G46050 | (pep) | FY(ph)VYRAEVTDSVDVK | 0.82 | | | | | |
| AT5G49890 | (-) | TTFGS(ph)QILR | 27.40 | 0.3061 | | | | |
| AT5G57350 | (ATP) | LGMGS(ph)NMYPS(ph)SSLLGK | 2.34 | | | 3.08 | 0.2003 | 1.42E-01 |
| AT5G60660 | (Aq) | ALGSFGSFGS(ph)FR | | | | -1.55 | | 8.73E-02 |
| AT5G64840 | (ABC) | NGAS(ph)GISSGVKLENIR | -23.26 | | | | | |
| AT5G67500 | (por) | IDTDSSVLTTVTLTEILPSTK | 3.24 | | | | | |

## others - amino acid metabolism

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G04710 | AGFY(ph)DIGIGAGLES(ph)M(ox)TT(ph)NPR | | | | 0.12 | 0.3530 | |
| AT1G22410 | DGVKLPS(ph)YR | -21.17 | | | | | |
| AT1G55880 | VKCFLIDPPGSGLY(ph)NK | -1.15 | | | | | |
| AT1G69523 | FNGGADVKKT(ph)S(ph)LSR | 1.32 | | | | | |
| AT2G14170 | RLYKEADDNT(ph)K | -42.99 | | | | | |
| AT2G20610 | RAVADY(ph)MNR | -20.98 | | | | | |
| AT2G41040 | (ac)AMAALT(ph)SSSSAIT(ph)LLNK | | | | 10.00 | | |
| AT2G43940 | (s)(t)KKFS(ph)TLPNAK | -21.35 | | | | | |
| AT3G04520 | (ac)MVTPT(ph)T(ph)IRTVDLRS(ph)DTVTK | | | | -0.66 | | |
| AT3G17365 | DKGVYILIT(ph)YGAPIYR | -0.23 | | | | | |
| AT3G53580 | (ac)M(ox)EIAAVS(ph)TVS(ph)VAPQSRR | | | | 0.19 | | |
| AT3G54640 | GTNLDS(ph)ILEMLDK | 1.33 | 0.0600 | | 1.01 | | |
| AT5G06300 | TLM(ox)PREIT(ph)GETIGEVK | -21.17 | | | -12.32 | | |
| AT5G48060 | IM(ox)S(ph)LLS(ph)GYFLVGK | | | | | | |
| AT5G63890 | MY(ph)SGV(s)LD(s)FLK | 0.19 | | | | | |

## others - cell wall.

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G09550 | KGS(ph)GYGSSTLMEK | 0.72 | | | | | |
| AT1G53840 | (ac)M(ox)DSVNS(ph)FKGYGK | | | | 10.00 | | |
| AT3G05610 | KT(ph)CEDTLIK | 1.50 | | | | | |

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT3G10710 | KVADIVVAKDGS(ph)GK | -43.38 | | | | | |
| AT3G14310 | T(ph)RT(ph)IITGSR | 7.58 | | | | | |
| AT3G19620 | DVKCGDQT(ph)LISAAVK | -20.31 | | | | | |
| AT3G61130 | FYLPEVY(ph)PK | 2.22 | | | | | |
| AT3G61130 | NDLLQELQARLKDS(ph)QR | 64.95 | | | | | |
| AT4G20460 | M(ox)LS(ph)FSRARSQGR | -20.63 | | | | | |
| AT4G30440 | PSIEDELFPST(ph)PGK | | | | -10.00 | | |
| AT4G35670 | GRLT(ph)EESAVAISNVKFVDFR | -2.37 | | | | | |
| AT4G37800 | M(ox)VVSLFS(ph)S(ph)R | 1.17 | | | 0.93 | 0.9872 | 1.00E+00 |
| AT5G06870 | TMTLFLLLS(ph)TLLLT(ph)T(ph)S(ph)LAK | | | | -0.61 | | |
| AT5G39280 | ISCART(ph)GGVK | -21.69 | | | | | |

## others - cell.

(c) cycle; (d) division

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G65920 | (d) | QVLVY(ph)NK | 0.34 | | | -0.24 | | |
| AT1G65920 | (d) | LS(ph)SVITIVR | 10.32 | | | | | |
| AT1G70620 | (c) | QDS(ph)NKPYGK | -14.29 | | | | | |
| AT2G41830 | (c) | GM(ox)GLPRS(ph)LSR | -19.45 | | | | | |
| AT3G13210 | (c) | EIFDRANT(ph)Y(ph)NK | -25.38 | | | | | |
| AT5G12350 | (d) | VGS(ph)SFDIKMDSLLPK | -0.37 | | | | | |
| AT5G25380 | (d) | IRMVESLDAS(ph)ASK | 5.07 | | | | | |
| AT5G45190 | (c) | SRNVDVGDALIS(ph)QS(ph)PK | 0.52 | 0.3831 | | 0.12 | | |

## others - DNA.

(r ) repair; (s) synthesis; (u) unspecified

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G03190 | (r ) | HLGT(ph)QAKILALGLSSR | -42.34 | | | | | |
| AT1G12370 | (r ) | FNVDSY(ph)ISYVK | 1.23 | | | | | |
| AT1G12700 | (s) | MMIKRS(ph)ITTNMK | 1.09 | | | | | |
| AT1G26840 | (s) | MDIS(ph)DIGRK | 10.97 | | | | | |
| AT1G58050 | (s) | KS(ph)SGFGKSR | -22.07 | | | | | |
| AT1G58050 | (s) | AALPIS(ph)EVK | 7.96 | | | | | |
| AT1G58050 | (s) | S(ph)LM(ox)GDFLLIILK | 26.66 | | | | | |
| AT1G66730 | (s) | MNLT(ph)KGT(ph)ISPGK | -2.83 | | | | | |
| AT1G77320 | (r ) | NT(ph)HDQT(ph)MVY(ph)DS(ph)SSR | | | | 1.56 | 0.2035 | |
| AT2G13720 | (u) | WTIKFY(ph)K | 1.73 | | | | | |
| AT2G38810 | (s) | (t)(t)AAAANKDSVK | 3.71 | | | | | |
| AT2G45810 | (s) | YLKIEVM(ox)VT(ph)TGGTSLRDDIMR | 27.50 | | | | | |
| AT2G45850 | (u) | GRPPGS(ph)GKK | 3.41 | | | | | |
| AT3G10270 | (r ) | GY(ph)IIYSSPWIETK | 2.92 | | | | | |
| AT3G10690 | (s) | RVPLS(ph)SFR | 12.87 | | | | | |
| AT3G20540 | (s) | ELQM(ox)EDREAWI(s)YS(ph)ALD(s)ISTLKLYESM(ox)K | -21.44 | | | | | |
| AT3G21430 | (u) | (ac)M(ox)APS(ph)RSKK | -0.20 | 0.2417 | | -0.44 | | |
| AT3G21430 | (u) | SS(ph)LGKPRR | -20.81 | | | | | |
| AT3G27970 | (u) | (ac)DYRSSM(ox)ESSET(ph)LR | -0.69 | | | -0.93 | | 1.00E+00 |
| AT3G27970 | (u) | LYT(ph)RMRY(ph)QK | 29.13 | | | | | |
| AT3G42170 | (u) | (ac)M(ox)EVYNDDTEM(ox)RS(ph)PETQPIK | | | | 0.10 | | |
| AT3G42670 | (u) | WHAQPSVLVM(ox)GY(ph)T(ph)SFLTLMR | | | | 1.13 | | |
| AT3G42670 | (u) | RRS(ph)GRPER | -0.07 | | | | | |
| AT3G53320 | (s) | SSLDIS(ph)KTQQEK | 0.70 | | | | | |
| AT4G02070 | (r ) | ETGS(ph)KDKVVK | -20.09 | | | | | |
| AT4G12740 | (s) | M(ox)VVAGT(ph)EGFPNQASS(ph)LM(ox)K | | | | 10.00 | | |
| AT4G30100 | (s) | KNVNHIS(ph)E(s)(s)GK | 28.03 | | | | | |
| AT5G04980 | (s) | T(ph)PS(ph)FGAGSMFFAKPSLKKISESFR | -0.35 | | | | | |
| AT5G28740 | (r ) | LASVLNDDKFY(ph)SIK | 1.27 | | | | | |
| AT5G37630 | (s) | FLVAGS(ph)VAANR | 1.13 | | | | | |
| AT5G41360 | (r ) | ILEAFKT(ph)S(ph)KT(ph)VNTVFLSK | 3.13 | | | | | |
| AT5G44635 | (s) | M(ox)QET(ph)SKEIPAGSLPR | 3.53 | | | | | |
| AT5G44800 | (s) | KFLDSLSSLPSK(s)(s)SR | -0.76 | | | | | |
| AT5G62410 | (s) | TY(ph)DLSLFLK | 0.27 | | | | | |

## others - gluconeogenesis / glyoxylate cycle.

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT2G42790 | IVT(ph)DDS(ph)KES(ph)DKLGQVATSNASR | 13.27 | | | | | |

## others - glycolysis.

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G68750 | DEDNNKLRDALLIT(ph)INGIAAGMR | -23.94 | | | | | |
| AT3G52930 | GILAADES(ph)T(ph)GT(ph)IGKR | 10.00 | | | 10.00 | | |
| AT4G24620 | DIS(ph)HADSKKELLK | 3.58 | | | | | |
| AT5G08570 | VLGSHAKSIM(ox)LMS(ph)K | -1.88 | | | | | |

## others - hormone metabolism.

(ABA) abscissic acid; (IAA) auxin; (BR) brasinosteroids; (CK) cytokinin; (ET) ethylene; (GA) gibberellic acid; (JA) jasmonate; (SA) salicylic acid

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G14020 | (IAA) | EVRS(ph)ESNITQAR | 0.19 | | | | | |
| AT1G15360 | (ET) | S(ph)PSPSLTCLR | -0.10 | | | | | |
| AT1G22460 | (IAA) | INEIHKT(ph)R | 0.41 | 1.4700 | | -0.01 | | |
| AT1G79460 | (GA) | GGVLT(ph)TVVDDFFDVGGSK | -0.21 | | | | | |
| AT2G01830 | (CK) | TNGNVHHKS(ph)PKLALFAT(ph)NITNSEFDR | -4.55 | | | -8.99 | | |
| AT2G17230 | (BR) | LPPRSLS(ph)LTSSK | 27.98 | | | | | |
| AT2G27150 | (ABA) | YIDISNIPEMS(ph)MIK | 1.56 | | | | | |
| AT2G37980 | (IAA) | M(ox)S(ph)AAGAS(ph)PLAVAPITAPT(ph)TTTRR | | | | 10.00 | | |
| AT2G44810 | (JA) | RAPM(ox)VT(ph)VIS(ph)FGGPR | | | | -10.00 | | |
| AT3G02260 | (IAA) | AAFDS(ph)VS(ph)KSVQTLQGLRR | -2.02 | | | | | |
| AT3G03850 | (IAA) | ILGGS(ph)LVKT(ph)SKAPPK | 4.62 | | | | | |
| AT3G19820 | (BR) | (ac)M(ox)SDLQT(ph)PLVR | | | | 0.12 | | |
| AT4G02780 | (GA) | M(ox)PYVNNNGY(ph)LELAK | -20.97 | | | | | |
| AT4G09530 | (IAA) | FVIPT(ph)TFLKSPSFQK | -1.55 | | 1.63E-01 | -2.90 | | 4.70E-02 |

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT4G39400 | (BR) | S(ph)IEDGGFSTIEM(ox)VDM(ox)SIK | 12.82 | | | 5.99 | | |
| AT5G13350 | (IAA) | IMFGS(ph)LYR | 1.47 | 0.0800 | | 1.28 | 0.6968 | |
| AT5G35750 | (CK) | (ac)SITCELLNLT(ph)S(ph)KK | | | | -0.09 | | |
| AT5G37970 | (SA) | Y(ph)VNYFIVLKR | 0.27 | 0.0700 | | 0.38 | | |
| AT5G43830 | (IAA) | VDS(ph)SQNWAGHI | -22.80 | 0.1497 | | | | |
| AT5G51190 | (ET) | VWLGT(ph)FDTAM(ox)EAARGY(ph)DK | -3.25 | | | | | |

## others - lipid metabolism.

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G01610 | SATTVPIES(ph)LK | 5.10 | | | | | |
| AT1G02390 | T(ph)LLDPLYISYALRKK | -22.70 | | | | | |
| AT1G04010 | LT(ph)FETALKLR | -0.11 | 0.0200 | | 0.50 | | |
| AT1G55320 | FPLYSRVVEAAPS(ph)K | -21.05 | | | | | |
| AT2G03770 | KVMFVMY(ph)EEM(ox)R | 1.68 | | | | | |
| AT2G19880 | (ac)MS(ph)TLDS(ph)IDAILFSLSR | | | | -10.00 | | |
| AT2G32260 | ETQRTEGI(s)(t)SDIIM(ox)RIVK | -20.61 | | | | | |
| AT2G42450 | RPS(ph)SVTSS(ph)R | -10.00 | | | -10.00 | | |
| AT3G03520 | Y(ph)FKILSAPANDDHPK | 11.57 | 0.3717 | | | | |
| AT3G11670 | RSSLEILS(ph)GFK | | | | -10.00 | | |
| AT3G23410 | QS(ph)IELVT(ph)KRGT(ph)IEAYIATR | -0.72 | | | | | |
| AT3G48610 | GAT(ph)SRFIRAS(ph)K | 1.20 | | | | | |
| AT3G48990 | RALSVS(ph)GKFNLTHAR | 29.28 | | | | | |
| AT3G51840 | ARET(ph)ASLGR | 26.98 | | | | | |
| AT4G04930 | SWSQVIY(ph)M(ox)YIMDTT(ph)VGPYSR | 0.48 | | | 0.24 | | |
| AT4G21540 | T(ph)LVYQGPDSK | 10.50 | | | 5.91 | | |
| AT4G28130 | Y(ph)KNLSDFLK | -1.48 | | | | | |
| AT4G36480 | ITLEKIM(ox)T(ph)K | 3.24 | | | | | |
| AT5G06090 | AVLPKFFM(ox)DDIS(ph)MDAWRAFGSCDK | 2.17 | | | | | |
| AT5G07010 | AIAELCS(ph)FENLKK | -23.97 | | | | | |
| AT5G40610 | FS(ph)SLSAAM(ox)SPALEK | -0.36 | | | | | |
| AT5G46290 | RGEADMMIAGGT(ph)EAAIIPIGLGGFVACRALS(ph)QR | 11.99 | | | | | |

## others - major CHO metabolism.

(d) degradation; (s) synthesis

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G47840 | (d) | T(ph)VVAMDGALY(ph)EK | 0.03 | 0.0300 | | -0.19 | | |
| AT1G35580 | (d) | SVLDT(ph)PLSSAR | -3.34 | 0.6871 | | -5.98 | | |
| AT1G35580 | (d) | FKLGEGVM(ox)PAS(ph)FK | 3.18 | 0.6871 | | 2.99 | | |
| AT1G12240 | (d) | (ac)M(ox)ASS(ph)DALLPIS(ph)AR | | | | 2.39 | | |
| AT3G43190 | (d) | T(ph)LMLNNR | 1.61 | | | 3.98 | 0.0561 | |
| AT3G43190 | (d) | Y(ph)IGDGVEFLNR | -20.42 | | | | | |
| AT1G77130 | (s) | (ac)M(ox)IPSS(ph)SPMESR | 0.60 | | | 0.36 | 0.2267 | 2.89E-01 |

## others - minor CHO metabolism.

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G06490 | DEDLIS(ph)DRER | -21.87 | | | | | |
| AT3G42850 | FEDS(ph)ISILVSSTVPEGK | 0.72 | | | | | |
| AT5G40390 | 108.66 SDS(ph)GINGVDFTEK b3/y11 . | 0.12 | 0.1942 | | | | |
| AT5G20250 | LT(ph)GIKENEKFK | 3.74 | | | | | |
| AT5G49650 | KGSFVPISNLY(ph)EGK | 0.25 | | | 0.11 | | |
| AT1G35910 | GQGFGILVS(ph)KIPK | 11.23 | | | | | |

## others - metal handling.

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT4G14030 | YGGPGY(ph)ATPLAAM(ox)S(ph)GPSEK | -0.31 | 0.7012 | 3.35E-01 | -1.53 | 1.0992 | 3.35E-01 |
| AT1G22990 | M(ox)HKV(t)V(s)GYVDPK | 2.31 | | | | | |

## others - misc.

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G01980 | SFY(ph)S(ph)YM(ox)APFVTKNPR | 10.00 | | | 10.00 | | |
| AT1G05240 | AEEIVRGVTVQY(ph)VSRQK | -19.85 | | | | | |
| AT1G07260 | ES(ph)YEAWVEIAEK | 2.05 | | | | | |
| AT1G10290 | S(ph)QQVSASALRHSLQDR | -21.97 | | | | | |
| AT1G16610 | AVQES(ph)LVLHVDSLSR | 0.09 | 0.1109 | | 0.26 | | |
| AT1G21100 | M(ox)LRLLAS(ph)Y(ph)SMVKCGK | | | | | | |
| AT1G22370 | (ac)M(ox)ADESS(ph)LDT(ph)K | | | | -10.00 | | |
| AT1G24110 | SLPGDAFDVITRIKT(ph)AVELK | -22.88 | | | | | |
| AT1G28580 | MAYPGS(ph)PILM(ox)K | -22.19 | | | | | |
| AT1G31550 | M(ox)AS(ph)LDS(ph)HVLMK | 5.39 | | | | | |
| AT1G45130 | T(ph)S(ph)HVQM(ox)VPSGSILY(ph)SVARY(ph)DEDIATYGNR | 28.19 | | | | | |
| AT1G52420 | DDAGS(ph)NKRT(ph)DVSLIK | 0.01 | | | 0.20 | | |
| AT1G52420 | M(ox)EEIRLS(ph)PLR | -45.27 | 0.0312 | | | | |
| AT1G52420 | LSPLRQTSVKSSLS(ph)GR | 2.43 | | | | | |
| AT1G55560 | RENYNLLDAVS(ph)R | 26.36 | | | 12.98 | | |
| AT1G55940 | S(ph)VSFQGVVDVK | -1.11 | | | | | |
| AT1G66540 | (ac)M(ox)VSAPYS(ph)EHWRNLR | 1.94 | | | 1.70 | 0.6302 | 1.68E-01 |
| AT1G69930 | LKFNT(ph)SIFK | 1.24 | 0.1900 | | 1.09 | | |
| AT1G74550 | AVMDEVY(ph)GGR | -1.56 | | | | | |
| AT1G80410 | AAY(ph)AEM(ox)LYILDPSK | 0.70 | | | | | |
| AT2G04060 | M(ox)LY(ph)SFWLK | -10.00 | | | -10.00 | | |
| AT2G14120 | S(ph)IKDALVAEEK | -0.34 | | | 0.42 | 0.3909 | |
| AT2G15480 | GAKS(ph)TLLTTPINAK | 3.69 | | | | | |
| AT2G16230 | ALEAVSLGGKIKVSTVHAM(ox)TVLGNSEPPSAGSFAPS(ph)YQAGLK | -10.00 | | | -10.00 | | |
| AT2G26600 | S(ph)LISSSKSAR | 11.43 | | | | | |
| AT2G30330 | (ac)MNT(ph)PMSLSAAR | | | | 10.00 | | |
| AT2G30330 | (ac)NT(ph)PM(ox)SLSAAR | 10.00 | | | 10.00 | | |
| AT2G30575 | EEQAVS(ph)QKT(ph)TVSSNAEVK | -1.01 | | | -1.00 | | |
| AT2G31432 | T(ph)QIIVDTGLLVIS(ph)GEDT(ph)RK | | | | 10.00 | | |
| AT2G37540 | (ac)M(ox)GIYGVMT(ph)GK | 1.45 | | 3.08E-01 | 0.18 | | |

| accession | pPeptide | replicate 1 log2(ahk1/wt) | replicate 1 sd | replicate 1 p-value | replicate 2 log2(ahk1/wt) | replicate 2 sd | replicate 2 p-value |
|---|---|---|---|---|---|---|---|
| AT2G46660 | (ac)MAT(ph)KLES(ph)SLIFALLS(ph)K | | | | -0.23 | | |
| AT3G01620 | YLLPGNCKRES(ph)G | 14.58 | | | | | |
| AT3G03640 | FS(ph)ISWSRIFPHGK | -1.63 | | | | | |
| AT3G21770 | RGLFQS(ph)DSALTT(ph)NS(ph)ATLK | 10.00 | | | 10.00 | | |
| AT3G22250 | LS(ph)GFGEKEVEDGLR | 2.46 | | | | | |
| AT3G24200 | M(ox)M(ox)FPLS(ph)LR | -1.76 | | | | | |
| AT3G46650 | LGGIE(s)MI(t)LNK | 29.28 | | | | | |
| AT3G46680 | DLPT(ph)SGVGPLDR | -20.70 | | | | | |
| AT3G61760 | YNDAY(ph)LRR | -10.00 | | 1.54E-01 | -10.00 | | |
| AT3G61880 | GLPFVGSM(ox)SLM(ox)(s)N(t)LAHR | -21.55 | | | | | |
| AT4G15330 | TEIMVVS(ph)RR | -4.02 | | | | | |
| AT4G16220 | EVAKAIAQDT(ph)TTDLPK | -21.23 | | | | | |
| AT4G19850 | (ac)M(ox)GIIWS(ph)IFSKT(ph)K | | | | 2.58 | | |
| AT5G03610 | ES(ph)LTASLIK | -19.31 | | | | | |
| AT5G04660 | FM(ox)LGKEDADIT(ph)GIS(ph)GVK | -1.13 | 0.0300 | | -1.99 | | |
| AT5G04885 | GVS(ph)TVM(ox)VSYSSWNGEK | -6.62 | | | | | |
| AT5G07360 | DADM(ox)KVVEVLGS(ph)K | 28.55 | | | | | |
| AT5G11130 | DY(ph)IS(ph)LIS(ph)NR | | | | | | |
| AT5G11340 | (ac)M(ox)GAGREVS(ph)VSLDGVR | 0.69 | | | 0.46 | | |
| AT5G14130 | DGLVS(ph)KASRVT(ph)GK | -20.67 | | | | | |
| AT5G19370 | LS(ph)IT(ph)PT(ph)LAIAS(ph)PPHLR | -1.88 | | | -2.12 | | 1.00E+00 |
| AT5G27410 | SLST(ph)T(ph)LM(ox)YSFAQR | | | | 1.17 | | |
| AT5G38450 | LGDLT(ph)IPK | 10.01 | | | | | |
| AT5G44620 | RPS(ph)QT(ph)TVVGGFTIPK | -23.16 | | | | | |
| AT5G45700 | YQIVVFT(ph)AGLR | -66.12 | | | | | |
| AT5G50400 | SSPDY(ph)MKTGNAVLK | 2.94 | | | | | |
| AT5G57920 | SVVDAPAPVNIVLS(ph)PNYNR | -23.74 | | | | | |
| AT5G58390 | DFARAM(ox)IKM(ox)GDIS(ph)PLTGSNGQIR | -1.62 | | | 1.35 | | |
| AT5G64100 | ISQAS(ph)DVNLPGPSDS(ph)VAKQK | -23.60 | | | | | |
| AT5G66060 | Y(ph)LIELAKPHMEK | -0.07 | 0.0300 | | -0.20 | | |
| AT5G67230 | S(ph)PLEIT(ph)VPS(ph)K | 10.00 | | | 10.00 | | |

## others - nucleotide metabolism.

| accession | pPeptide | replicate 1 log2(ahk1/wt) | replicate 1 sd | replicate 1 p-value | replicate 2 log2(ahk1/wt) | replicate 2 sd | replicate 2 p-value |
|---|---|---|---|---|---|---|---|
| AT1G27450 | SDAEY(ph)FAKAAASR | -20.55 | | | | | |
| AT1G63660 | SLNVFSLVISGT(ph)SSLK | 10.00 | | | 10.00 | | |
| AT1G68720 | NLIRS(ph)PPIK | -1.63 | 0.6227 | | | | |
| AT1G79470 | (ac)M(ox)ST(ph)LEDGFPADK | | | | -2.03 | | |

## others - protein.

(a) amino acid activation; (as) (f) folding; (g) glycosylation; (s) synthesis; (t) targeting

| accession | category | pPeptide | replicate 1 log2(ahk1/wt) | replicate 1 sd | replicate 1 p-value | replicate 2 log2(ahk1/wt) | replicate 2 sd | replicate 2 p-value |
|---|---|---|---|---|---|---|---|---|
| AT1G01100 | (s) | KDEPAEESDGDLGFGLFD | -0.55 | 0.7607 | | 0.59 | | |
| AT1G07910 | (s) | KPQS(ph)IM(ox)LADK | 29.54 | | | | | |
| AT1G09270 | (t) | MS(ph)LRPSTRAELR | 4.45 | | | | | |
| AT1G13020 | (s) | SSGPPGRMS(ph)R | -19.12 | | | | | |
| AT1G18130 | (a) | LFAILLEQY(ph)KGK | -0.11 | 0.0400 | | -0.01 | | |
| AT1G36390 | (f) | DRLS(ph)TESNAK | -61.29 | | | | | |
| AT1G53880 | (s) | VVFEILKT(ph)SAQNKK | -21.42 | | | -10.00 | | |
| AT1G57620 | (t) | T(ph)GIAAKDWDSIARK | -2.88 | | | | | |
| AT1G64790 | (s) | ALLEGGS(ph)DDEGASTEAQGR | | | | 0.95 | | |
| AT1G65590 | (g) | IAGVLPLFM(ox)LFIAGTIS(ph)AFEDIER | -21.30 | | | -2.89 | | |
| AT1G68660 | (s) | (ac)M(ox)ET(ph)AICGRLALAPS(ph)SLFNSK | | | | 0.47 | | |
| AT1G71350 | (s) | KGS(ph)AY(ph)PS(ph)EIHKK | 0.78 | | | | | |
| AT2G07734 | (s) | RSFYKEIS(ph)VEK | -43.03 | | | | | |
| AT2G14720 | (t) | SLGIDS(ph)RKIDK | 1.52 | 0.1400 | | 0.31 | | |
| AT2G17790 | (t) | LY(ph)LLCT(ph)AGSVYIK | -1.05 | | | | | |
| AT2G18020 | (s) | T(ph)GRLRGQAAASAAK | 26.10 | | | | | |
| AT2G19720 | (s) | VNDCKALT(ph)Y(ph)RQDVK | -0.45 | | | 0.23 | 0.4629 | |
| AT2G25840 | (a) | INLLDS(ph)KDLIVDKIK | 61.65 | | | | | |
| AT2G27710 | (s) | EESDDDM(ox)GFSLFE | 0.86 | | | 0.12 | | |
| AT2G27710 | (s) | EEKEES(ph)DDDM(ox)GFSLFE | 0.48 | | | | | |
| AT2G33210 | (f) | M(ox)IS(ph)TSEEIAQVGTISANGDR | -0.54 | | | | | |
| AT2G33840 | (a) | ADKY(ph)WPLVMDIAR | -18.43 | | | | | |
| AT2G36070 | (t) | FSAAT(ph)EEVKESFK | -1.12 | | | | | |
| AT2G36620 | (s) | SQVKGNIPKS(ph)AAPK | -20.29 | | | | | |
| AT2G42650 | (s) | SAVS(ph)GKPDIVKS(ph)K | 5.06 | | | | | |
| AT2G42710 | (s) | GTLALPHS(ph)VKKDVK | 3.35 | | | | | |
| AT2G47020 | (s) | LINQPEYSPEEFS(ph)RANK | 1.20 | | | | | |
| AT2G47840 | (t) | MAS(ph)LCLSLHQT(ph)LT(ph)NPLSAPR | -10.00 | | | -10.00 | | |
| AT3G01175 | (s) | RFELIS(ph)FFQVPR | -0.16 | | | | | |
| AT3G02530 | (f) | AT(ph)LQFLDTFKTPVVMGDEPDK | -21.43 | | | | | |
| AT3G05560 | (s) | ITVTADGQFS(ph)K | -21.16 | 0.1410 | | | | |
| AT3G09200 | (s) | EESDEEDYGGDFGLFDEE | | | | 0.74 | 1.0830 | |
| AT3G09200 | (s) | VEEKEES(ph)DEEDYGGDFGLFDEE | -1.09 | 0.2035 | | | | |
| AT3G11250 | (s) | KEESDEEDYEGGFGLFDEE | | | | 0.84 | | |
| AT3G12340 | (f) | KVSILYT(ph)GK | 28.49 | | | -3.34 | | |
| AT3G48820 | (g) | WVPS(ph)RSTIRSAR | 2.31 | | | | | |
| AT3G58140 | (a) | (ac)T(ph)VFS(ph)VQSTIFSRASVALLS(ph)SNGFKR | | | | -10.00 | | |
| AT4G01800 | (t) | LETEISALSDS(ph)ELR | 30.32 | | | | | |
| AT4G14455 | (t) | MDS(ph)ARGIMS(ph)GTINR | -22.78 | | | | | |
| AT4G25890 | (s) | KKEESEEEEGDFGFDLFG | 0.43 | | | | | |
| AT4G26300 | (a) | FSADHLTFT(ph)T(ph)VT(ph)EK | | | | 10.00 | | |
| AT4G36420 | (s) | GVS(ph)KEEAEKIIEK | 5.11 | | | | | |
| AT5G05670 | (t) | TDKLT(ph)AHTKEFIR | -23.06 | | | | | |
| AT5G19620 | (t) | T(ph)S(ph)PETILRQLTTK | 1.77 | | | | | |
| AT5G20200 | (t) | HKEAEVGT(ph)LTVPTHS(ph)SQIART(ph)ILDHLER | -10.00 | | | -10.00 | | |
| AT5G27640 | (s) | DGEVSDVEEDEYEAK | 1.23 | 0.1131 | | 1.80 | | |
| AT5G47700 | (s) | KKDEPAEESDGDLGFGLFD | | | | -0.10 | | |
| AT5G47700 | (s) | KDEPAEESDGDLGFGLFD | 1.45 | 0.0809 | | 0.59 | | |
| AT5G47700 | (s) | DEPAEESDGDLGFGLFD | | | | 10.00 | | |
| AT5G53530 | (t) | KTY(ph)PFEFSSVEMPYETYNGVNVR | 10.26 | 0.4368 | | | | |
| AT5G66030 | (t) | SLEVKLDS(ph)T(ph)VAR | -19.88 | | | | | |
| ATCG00860 | (as) | IKS(ph)LM(ox)IPS(ph)YM(ox)IELR | -4.54 | | | | | |

## others - photosynthesis

(CC) calvin cycle; (LR) light reaction

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G05385 | (LR) | T(ph)KSGKELPK | 29.43 | | | | | |
| AT2G21170 | (CC) | LVSSSSSS(ph)HR | -22.11 | | | | | |
| AT2G21170 | (CC) | GPEFATIVN(s)VT(s)K | -20.82 | | | | | |
| AT2G39730 | (CC) | GLAYDTSDDQQDITR | | | | -10.00 | | |
| AT2G46820 | (LR) | AT(ph)TEVGEAPATTTEAETTELPEIVK | -23.07 | 0.3715 | 2.23E-01 | -0.71 | 0.3240 | 2.23E-01 |
| AT2G46820 | (LR) | AT(ph)TEVGEAPATTTEAETTELPEIVK | -0.48 | 0.3715 | 2.23E-01 | -0.71 | 0.3240 | 2.23E-01 |
| AT2G47400 | (CC) | ATSEGEIS(ph)EKVEK | -3.52 | | | | | |
| AT3G21055 | (LR) | VPSAT(ph)GGRR | -1.83 | | | | | |
| AT3G21740 | (LR) | Y(ph)VGANALM(ox)AWEK | -21.32 | | | | | |
| AT4G02630 | (LR) | NKSLES(ph)S(ph)SKSNHTIVPVVS(ph)K | | | | | | |
| AT5G01530 | (LR) | NLAGDVIGT(ph)RTEAADAK | -23.03 | 0.2282 | | | | |
| AT5G51545 | (LR) | (ac)M(ox)ALQIHSPCS(ph)FSTR | -10.00 | | | -10.00 | | |
| ATCG00020 | (LR) | (ac)T(ph)AILERR | 0.78 | 0.1930 | 4.54E-01 | 0.93 | 0.9974 | 8.03E-01 |
| ATCG00270 | (LR) | (ac)T(ph)IALGKFTK | 1.33 | 0.2712 | 4.72E-01 | 0.10 | 0.3942 | 7.19E-01 |

## others - redox.

| | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G21750 | SASGNVVVYEGDR | -21.39 | | | -10.00 | | |
| AT1G63940 | M(ox)ALAS(ph)T(ph)T(ph)LPTK | | | | -0.68 | | |
| AT1G77510 | T(ph)EETAAKDEL | 10.28 | | | | | |
| AT2G04700 | FSEQY(ph)ARR | 10.00 | | | 10.00 | | |
| AT3G10130 | M(ox)EM(ox)T(ph)TPVIT(ph)S(ph)KAK | | | | -1.38 | | |
| AT3G10130 | EKM(ox)EMT(ph)TPVVTRK | -0.26 | | | | | |
| AT3G52960 | NRRT(ph)NS(ph)ASATTR | 2.36 | | | | | |
| AT3G56420 | LVGAET(ph)SELQK | 1.16 | | | | | |
| AT4G23100 | S(ph)MLLHSVKRSK | 7.95 | | | -0.74 | | |
| AT4G23100 | VGT(ph)LGLDMMLR | -20.04 | | | | | |
| AT4G35460 | DVLGGLKVKNVVT(ph)GDVS(ph)DLK | 3.70 | | | | | |
| AT4G39830 | S(ph)MS(ph)EKATGLASIPFK | -1.05 | | | | | |
| AT5G06690 | KIMAALAS(ph)NPQM(ox)LTR | -23.65 | | | | | |
| AT5G39865 | Y(ph)LGGVEEIKK | 1.78 | | | 4.89 | 0.1105 | |

## others - RNA

(p) processing; (b) binding; (t) transcription

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G14220 | (p) | S(ph)QISDLVS(ph)S(ph)LKK | -10.00 | | | -10.00 | | |
| AT1G28090 | (p) | (ac)M(ox)IEDFS(ph)GARGSNK | | | | -10.00 | | |
| AT1G30590 | (p) | IDKKDPS(ph)IVTYVDNLLR | -21.37 | | | -0.91 | | |
| AT1G43190 | (p) | I(s)F(s)QLQTI | 3.08 | | | | | |
| AT1G54590 | (p) | M(ox)VVS(ph)KSTNSR | 10.30 | | | | | |
| AT2G24120 | (t) | S(ph)AKHM(ox)LIPY(ph)VPMLVPPK | -0.84 | | | | | |
| AT2G25850 | (p) | FISKIT(ph)ELR | 28.96 | | | | | |
| AT3G07750 | (p) | GGEEL(s)(s)ELALALQR | -1.18 | | | | | |
| AT3G07750 | (p) | IGGT(ph)DVIASVK | 1.91 | | | | | |
| AT3G13224 | (b) | ILPT(ph)S(ph)LPNVRSLSQ | -1.66 | | | | | |
| AT4G11130 | (t) | SSFRKLS(ph)LR | -22.03 | | | | | |
| AT4G21710 | (t) | M(ox)LARAS(ph)AK | -21.08 | | | | | |
| AT4G32850 | (p) | SVSISGT(ph)DSPLLPS(ph)R | 1.78 | | | | | |
| AT4G35785 | (b) | S(ph)RS(ph)LPRPVSPSRSR | -22.90 | 0.0070 | | | | |
| AT5G02250 | (p) | KYT(ph)ALVLR | -19.82 | | | | | |
| AT5G09920 | (t) | MLNDLS(ph)LVK | -21.78 | | | | | |
| AT5G19030 | (b) | ALS(ph)SSTFQIPFLK | -3.58 | | | | | |
| AT5G26742 | (p) | GSS(ph)DDWLIGGRS(ph)S(ph)S(ph)SS(ph)RAPS(ph)R | | | | | | |
| AT5G46420 | (p) | VS(ph)ECNLSVSR | 10.72 | | | | | |
| AT5G46870 | (b) | M(ox)S(ph)M(ox)VT(ph)VKVSNVSLEATER | -1.62 | | | | | |
| AT5G51410 | (b) | SPHGRS(ph)GHRR | -22.08 | | | | | |
| AT5G59950 | (b) | (ac)STGLDMS(ph)LDDM(ox)IAKNRK | -1.48 | 0.0495 | | -0.72 | | 2.24E-02 |
| AT5G61140 | (p) | TLRQVE(s)(t)QT(ph)MIR | 2.75 | | | | | |

## others

(C1) C1 metabolism; (Co) Co-factor and vitamine metabolism; (f) fermentation (mit) mitochondrial electron transport; (mi) micro RNA; (OPP) OPP; (PAM) polyamine metabolism; (sec) secondary metabolism; (TCA) TCA/org transformation; (tet) tetrapyrrole synthesis; (na) not assigned

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G03630 | (tet) | GLASGLNGQNSSM(ox)IDGGEFDGAK | -23.18 | | | | | |
| AT1G03830 | (na) | DVRLQM(ox)S(ph)LLNEK | -23.06 | | | | | |
| AT1G05060 | (na) | MS(ph)FLGAGRLAGK | 5.09 | | | | | |
| AT1G08070 | (na) | LKGVT(ph)NASSLR | 0.98 | | | | | |
| AT1G08430 | (na) | EMS(ph)ISLKQM(ox)IK | 8.39 | | | | | |
| AT1G08580 | (na) | AIHGDPLT(ph)NK | -19.70 | | | | | |
| AT1G09450 | (na) | M(ox)EKYES(ph)AKEAVSDPFFSDM(ox)LM(ox)DQIS | 11.18 | | | | | |
| AT1G10450 | (na) | Y(ph)ESFLELMK | 1.54 | 0.0500 | | 2.39 | | |
| AT1G10500 | (sec) | (ac)M(ox)AFATGITTSS(ph)NPTFLGLK | 0.21 | | | 1.83 | 0.2233 | 9.04E-01 |
| AT1G10690 | (na) | WVIAGIPS(ph)RSPLK | 3.83 | | | | | |
| AT1G11320 | (na) | M(ox)DPIAS(ph)VLEKVKSFT(ph)K | -21.86 | | | | | |
| AT1G11770 | (sec) | AKS(ph)FY(ph)SY(ph)M(ox)APFVTKNPR | 0.73 | | | | | |
| AT1G11940 | (na) | YS(ph)VKMSPVIPEEK | 3.92 | | | | | |
| AT1G12150 | (na) | KET(ph)EAAMIAAEEAEKR | 1.17 | 0.1800 | | 1.10 | | |
| AT1G13630 | (na) | FKELQVILEQLLQEEGT(ph)FRK | -23.23 | | | | | |
| AT1G15280 | (na) | QSNNPS(ph)KQPR | -25.05 | 0.1075 | | | | |
| AT1G15370 | (na) | RLIRLKPPS(ph)EV | -22.31 | | | | | |
| AT1G15740 | (na) | I(t)DIGI(s)YLK | 1.26 | | | | | |
| AT1G15760 | (na) | KNS(ph)PPLTSR | -19.14 | | | | | |
| AT1G15940 | (na) | EDLT(ph)KSNVKK | 1.69 | | | | | |
| AT1G17940 | (na) | Y(ph)LTEKIPK | 3.52 | | | | | |
| AT1G19140 | (na) | DVGVS(ph)PSIVGSFSR | -21.78 | | | | | |
| AT1G19360 | (na) | MLKS(ph)DFVTLSEK | -20.42 | | | | | |
| AT1G20100 | (na) | ENVSSAKS(ph)SYKTDEDT(ph)IM(ox)SLQT(ph)SR | -0.38 | | | 0.38 | 1.0086 | 3.07E-01 |
| AT1G20970 | (na) | T(ph)QNRDALRADIQK | 2.31 | 0.3579 | | | | |
| AT1G21323 | (na) | VSY(ph)ETLDRLT(ph)AR | 4.49 | | | | | |
| AT1G23790 | (na) | LDPGT(ph)PVPIIK | 1.24 | 0.1100 | | -0.97 | 0.3144 | 3.11E-01 |

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT1G23970 | (na) | T(ph)PFIPHFHGGALGDGIFK | 0.66 | | | | | |
| AT1G24180 | (TCA) | ESPIPDASELFTNM(ox)YVK | -21.86 | | | | | |
| AT1G27752 | (na) | FNHET(ph)S(ph)IPAVT(ph)K | 10.00 | | | 10.00 | | |
| AT1G27840 | (na) | T(ph)LQS(ph)KQTGSQSVK | -0.63 | | | | | |
| AT1G29240 | (na) | VHS(ph)PVHPSNGK | -19.25 | | | | | |
| AT1G30140 | (na) | NYM(ox)S(ph)RLK | -0.98 | 0.3000 | | -0.31 | | |
| AT1G31660 | (na) | Y(ph)EILKEDK | 1.76 | | | | | |
| AT1G31910 | (sec) | TTCl(t)(s)GVSSIHLE | -2.59 | | | | | |
| AT1G32100 | (sec) | (ac)M(ox)GES(ph)KRTEK | 1.15 | | | -0.09 | 0.4979 | 3.94E-01 |
| AT1G32120 | (na) | FES(ph)LRTAR | -4.20 | | | | | |
| AT1G33350 | (na) | S(ph)HPET(ph)EEIY(ph)M(ox)ILDSLISF | | | | -1.01 | | |
| AT1G33400 | (na) | DAFRDIT(ph)VSMSLESSLVGK | 0.07 | | | | | |
| AT1G34070 | (na) | LSLY(ph)GTLT(ph)PK | 3.61 | | | | | |
| AT1G34350 | (na) | LAHT(ph)KEE | 29.07 | | | | | |
| AT1G35190 | (sec) | T(ph)EM(ox)LGKPIATMR | -2.38 | | | | | |
| AT1G35220 | (na) | S(ph)HAVTLYEAGK | 6.04 | | | 2.91 | | |
| AT1G35430 | (na) | (ac)M(ox)LTDVFWLRT(ph)R | | | | -0.99 | | |
| AT1G42430 | (na) | (ac)S(ph)EM(ox)AAS(ph)SAISLLDIK | | | | -10.00 | | |
| AT1G43010 | (na) | T(ph)VDKAEATFK | 0.19 | | | | | |
| AT1G43010 | (na) | M(ox)RDYSVLLS(ph)S(ph)YT(ph)KPVR | | | | | | |
| AT1G44170 | (f) | M(ox)FY(ph)QQRVLVS(ph)LLR | | | | | | |
| AT1G45545 | (na) | ARHLSAVS(ph)ELGT(ph)IR | -20.06 | | | | | |
| AT1G48560 | (na) | NKLDSSIIS(ph)AK | 1.81 | | | | | |
| AT1G48880 | (na) | ALSSLAIESPRNSSSSVF(t)(s)PIG(s)AFASPR | -22.85 | | | | | |
| AT1G50120 | (na) | CLEVS(ph)VT(ph)LET(ph)LETINR | 10.00 | | | 10.00 | | |
| AT1G50790 | (na) | DDEFIS(ph)FAR | -21.45 | | | | | |
| AT1G51350 | (na) | M(ox)PT(ph)TTTSSASSSSSASGNNR | 10.91 | | | | | |
| AT1G51630 | (na) | FEDIY(ph)DVDKLIK | 2.00 | | | | | |
| AT1G51900 | (na) | DFEPT(ph)LPDYDQVITR | -21.21 | | | | | |
| AT1G53260 | (na) | GT(ph)T(ph)T(ph)AKHEPELSGT(ph)S(ph)T(ph)AK | -20.98 | | | | | |
| AT1G53280 | (na) | S(ph)FSISATMSSSTKK | 2.18 | | | | | |
| AT1G61240 | (na) | S(ph)SM(ox)IPSLLQRK | -23.19 | | | | | |
| AT1G61690 | (na) | SQIHAEKGNAET(ph)MSGFR | -20.14 | | | | | |
| AT1G61690 | (na) | SDPLNS(ph)RPGKT(ph)AEAQGAY(ph)EVR | -18.26 | | | | | |
| AT1G61720 | (sec) | S(ph)CLKSKSVK | 0.83 | | | | | |
| AT1G62420 | (na) | NGY(ph)M(ox)QAKWFGFYK | -0.16 | 0.0700 | | 0.89 | | |
| AT1G65230 | (na) | (ac)M(ox)TTS(ph)SFLLPAS(ph)PPSAAFLRRR | | | | -10.00 | | |
| AT1G65710 | (na) | M(ox)IESVKPNS(ph)RTSR | -22.44 | | | | | |
| AT1G67120 | (na) | VRIS(ph)SDLGEK | -21.85 | | | | | |
| AT1G67120 | (na) | M(ox)AIDGS(ph)FNLK | -20.27 | | | | | |
| AT1G67540 | (na) | S(ph)GSVSEEEEEKRNLGTEK | 2.27 | | | | | |
| AT1G68780 | (na) | NFLS(ph)GALPLSVGGLYSLLK | -0.20 | 0.0400 | | 0.12 | | |
| AT1G69280 | (na) | KS(ph)NLSFDSSLHNK | -1.44 | | | | | |
| AT1G69680 | (na) | NIS(ph)AVAT(ph)TAIGEMAISKGR | 0.47 | | | | | |
| AT1G70180 | (na) | DQS(ph)PPRNAGRVTGSPR | -20.49 | | | | | |
| AT1G70270 | (na) | YKS(ph)M(ox)VKQTM(ox)NK | 0.33 | | | | | |
| AT1G70770 | (na) | M(ox)TAIDSDDDGVVR | -53.41 | 0.0136 | | | | |
| AT1G70770 | (na) | SNGYGDDGYDFDGSDDEIATLK | -21.96 | 0.0136 | | | | |
| AT1G71460 | (na) | LVHM(ox)Y(ph)T(ph)ACGSVK | 10.00 | | | 10.00 | | |
| AT1G72020 | (na) | SVS(ph)KIIASSEASVSR | -19.19 | | | | | |
| AT1G72270 | (na) | T(ph)FDFHGFPKQAPRR | -1.67 | | | | | |
| AT1G72270 | (na) | DAIAENLS(ph)R | 0.53 | | | | | |
| AT1G72450 | (na) | VET(ph)S(ph)ETRPFK | -44.03 | 0.0154 | | | | |
| AT1G73480 | (na) | M(ox)ICS(ph)SKGT(ph)ILIARGK | 0.44 | | | 0.21 | 0.7467 | 9.62E-01 |
| AT1G74140 | (na) | T(ph)LGPLY(ph)LLK | -1.34 | | | | | |
| AT1G74530 | (na) | (ac)MATST(ph)TSAIR | | | | -0.72 | | |
| AT1G74770 | (na) | M(ox)SQKVS(ph)QFGPSKK | 2.37 | | | | | |
| AT1G76780 | (na) | Y(ph)AKQNKIQEVM(ox)NDEDK | -2.79 | | | | | |
| AT1G78480 | (sec) | RM(ox)RY(ph)GEEFL(s)(s)R | -2.68 | | | | | |
| AT1G78640 | (na) | HLLPEDS(ph)QKIDK | 0.83 | | | | | |
| AT1G79750 | (TCA) | M(ox)IS(ph)LT(ph)PSLFLNK | | | | -2.09 | 0.0377 | |
| AT1G80280 | (na) | LS(ph)S(ph)EM(ox)VLPT(ph)QNALSLLK | 1.38 | | | -0.78 | 0.3049 | 1.85E-02 |
| AT1G80310 | (na) | FNYDTATLKPSSS(ph)PR | 28.02 | | | | | |
| AT1G80770 | (na) | (ac)MVM(ox)PS(ph)IDLYAS(ph)ALRKSK | | | | 0.98 | | |
| AT1G80910 | (na) | (ac)M(ox)GMASM(ox)S(ph)SGTESLR | | | | 10.00 | | |
| AT1G80970 | (na) | GRS(ph)VWVAWFK | -22.04 | | | | | |
| AT2G01340 | (na) | (ac)M(ox)GNS(ph)LGGKKTTK | -0.21 | | | 0.12 | 0.4337 | 4.07E-01 |
| AT2G03030 | (na) | Y(ph)EQRGKDLK | 2.57 | | | | | |
| AT2G03350 | (na) | LKGKPLKS(ph)LPDLLK | -1.91 | | | | | |
| AT2G04930 | (na) | IY(ph)AKAILEMIDPK | -19.06 | | | | | |
| AT2G05600 | (na) | PLTPLTIS(ph)KDT(ph)NKVILS(ph)LT(ph)S(ph)QER | | | | | | |
| AT2G07190 | (na) | M(ox)IKDFWS(ph)RLLK | -3.43 | | | | | |
| AT2G07640 | (na) | TREY(ph)MPLYKLGEK | 1.16 | | | 0.31 | | |
| AT2G08986 | (na) | T(ph)HMGFGYTMKALRS(ph)K | -1.67 | | | | | |
| AT2G11890 | (na) | LT(ph)T(ph)LLTPYHLKTLHQR | 0.39 | | | | | |
| AT2G16005 | (na) | (ac)M(ox)AISHTQLLLLLLVS(ph)LFFSPALCGPK | -10.00 | | | -10.00 | | |
| AT2G16500 | (PAM) | HS(ph)GHFGSTSGEKGKFGLTTVQILR | -19.59 | | | | | |
| AT2G18520 | (na) | FSTATGIDS(ph)Q(t)(t)AYPGAITMSK | 5.84 | | | | | |
| AT2G19710 | (na) | T(ph)LPPYRM(s)(s)ASSKAEK | -20.18 | | | | | |
| AT2G20020 | (na) | NYNHRT(ph)RPR | -21.15 | | | | | |
| AT2G20635 | (na) | ANLSS(ph)LQNY(ph)SRNK | 2.54 | | | | | |
| AT2G20690 | (Co) | (ac)M(ox)AART(ph)HCINLIPK | | | | 2.27 | | |
| AT2G22122 | (na) | (ac)S(ph)SM(ox)GANYAQLQVMQK | -10.00 | | | -10.00 | | |
| AT2G23360 | (na) | LIVIKT(ph)ELAGSGKR | -19.33 | | | | | |
| AT2G23370 | (na) | LMDLDAY(ph)LLR | 0.40 | | | | | |
| AT2G23630 | (na) | ALPPPDGLLINGAS(ph)K | 28.43 | | | | | |
| AT2G25050 | (na) | MGAPT(ph)SSLVLK | 3.42 | | | | | |
| AT2G25260 | (na) | GKY(ph)FFPILM(ox)T(ph)LSLFLIIR | -23.00 | | | | | |
| AT2G26050 | (na) | M(ox)CE(s)(s)NKVR | -21.01 | | | | | |
| AT2G26550 | (tet) | (ac)MASLLRPT(ph)PLLS(ph)T(ph)PR | | | | 0.74 | | |
| AT2G27285 | (na) | S(ph)KSLEPLEAEQAVSEK | -14.10 | | | | | |
| AT2G28580 | (na) | LFEPFS(ph)SNVNTK | 2.38 | | | | | |
| AT2G29190 | (na) | LKGS(ph)S(ph)NVLGGVGDR | -23.78 | | | | | |
| AT2G31110 | (na) | IRKPVHLLDLT(ph)T(ph)LSEY(ph)RK | -0.32 | | | | | |
| AT2G31270 | (na) | LFFDS(ph)SSSSPSKRK | -2.18 | | | | | |
| AT2G31270 | (na) | M(ox)S(ph)TPGSSRSIPFKSK | 0.00 | | | | | |
| AT2G31890 | (na) | FNQKIT(ph)S(ph)S(ph)FQK | -1.86 | | | | | |
| AT2G32880 | (na) | FIT(ph)HTQLK | 4.29 | | | | | |
| AT2G33400 | (na) | GGRSKGS(ph)S(ph)AIDK | -21.91 | | | | | |

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT2G33490 | (na) | S(ph)QLLS(ph)KPLITNS(ph)AS(ph)PLPIPPAITR | 2.17 | | | | | |
| AT2G35460 | (na) | T(ph)Y(ph)Y(ph)S(ph)HGR | | | | 1.44 | | |
| AT2G36330 | (na) | T(ph)M(ox)PS(ph)MSPSSVSTEK | -14.17 | | | | | |
| AT2G36560 | (na) | SNST(ph)PCIT(ph)PFPVT(ph)K | 0.32 | | | 0.08 | | |
| AT2G37230 | (na) | Y(ph)FNKMVSEGVEPTR | 4.71 | | | | | |
| AT2G37780 | (na) | VSIQGMFY(ph)K | -19.97 | | | | | |
| AT2G37940 | (na) | RFCS(ph)EISTEIGLLAENWK | -3.27 | | | | | |
| AT2G38320 | (na) | M(ox)VY(ph)VGDSLNR | 1.72 | | | | | |
| AT2G39190 | (na) | RFT(ph)LDLEYALGEVK | 0.24 | 0.0300 | | -0.40 | 0.1448 | |
| AT2G40150 | (na) | KS(ph)LNQTGSLTVFK | 30.23 | | | | | |
| AT2G40316 | (na) | FILIT(ph)LLFIS(ph)S(ph)R | | | | -0.01 | | |
| AT2G40960 | (na) | S(ph)VEERKEDY(ph)DR | -10.00 | | | -10.00 | | |
| AT2G41960 | (na) | SVFKSDS(ph)DLDVSK | -20.23 | | | | | |
| AT2G42480 | (na) | LDS(ph)LKSKLDEISLER | -0.18 | | | | | |
| AT2G42760 | (na) | DKENLNGKS(ph)R | -0.22 | | | | | |
| AT2G43255 | (na) | Y(ph)ADEHTM(ox)GVDTKK | -3.59 | | | | | |
| AT2G44200 | (na) | NRS(ph)PKGGVER | -19.27 | 0.1900 | | -3.40 | | |
| AT2G44200 | (na) | S(ph)EAG(s)(s)ASEKPDQS(ph)APGALFEEK | 27.60 | | | | | |
| AT2G44240 | (na) | ET(ph)KAT(ph)QVWQK | 1.91 | | | | | |
| AT2G44380 | (na) | MKS(ph)LMK | 3.04 | | | | | |
| AT2G45900 | (na) | IIIGNNGVFET(ph)K | -14.68 | | | | | |
| AT2G46470 | (mit) | SSVLNQRIRT(ph)LER | -6.46 | | | | | |
| AT2G47230 | (na) | RSLNLEKSAET(ph)LTK | -22.60 | | | | | |
| AT2G47510 | (TCA) | LS(ph)GGT(ph)TVTALR | -1.46 | | | | | |
| AT2G48060 | (na) | SMWTVLDY(ph)LR | 2.09 | | | | | |
| AT3G01810 | (na) | VLGLSS(ph)KNRR | 28.27 | | | | | |
| AT3G02650 | (na) | EVLKLM(ox)ES(ph)R | 4.23 | | | | | |
| AT3G04560 | (na) | RT(ph)NEDPDAEENKR | -22.59 | | | | | |
| AT3G04820 | (na) | IPM(ox)NKPVEKVGS(ph)TEEIEDES(ph)MK | -21.37 | | | | | |
| AT3G04960 | (na) | M(ox)RKET(ph)ELM(ox)ETSLK | 0.18 | 0.1038 | | -1.71 | | |
| AT3G05070 | (na) | ASEDES(ph)IEQKAAAR | -0.62 | | | | | |
| AT3G05340 | (na) | RM(ox)KAMGVT(ph)K | -9.53 | | | -10.00 | | |
| AT3G07530 | (na) | LISGEPS(ph)FGHLK | -21.49 | | | | | |
| AT3G07640 | (na) | KAS(ph)LHYVQVATETET(ph)S(ph)R | | | | -10.00 | | |
| AT3G08820 | (na) | (ac)M(ox)SIVTVPS(ph)AT(ph)S(ph)K | | | | 0.58 | | |
| AT3G10420 | (na) | VGDFS(ph)DDNRSGIDRSLHR | -0.04 | | | | | |
| AT3G11350 | (na) | SEKKY(ph)LDR | -1.50 | | | | | |
| AT3G11750 | (C1) | NLLETVAELIAS(ph)K | 0.76 | | | | | |
| AT3G13857 | (na) | FSRS(ph)ISSSSVLR | -20.58 | | | | | |
| AT3G14190 | (na) | ALNDITNKS(ph)GIHAKAAAS(ph)S(ph)K | | | | | | |
| AT3G14870 | (na) | FT(ph)ELRSM(ox)KPR | 0.42 | | | | | |
| AT3G15040 | (na) | QLQRSKS(ph)GLK | 29.46 | | | | | |
| AT3G15110 | (na) | AT(ph)T(ph)QPGDSSSGDNSEVNKSNEDQS(ph)SGD | -21.20 | 0.0605 | | | | |
| AT3G18420 | (na) | SAILIGAAVSM(ox)T(ph)GK | 0.92 | | | | | |
| AT3G18779 | (na) | (ac)M(ox)TVTCS(ph)IRVLHDSSVFLVVNLNR | 0.44 | | 2.36E-01 | 0.21 | | |
| AT3G19085 | (na) | DFLIGIS(ph)SVR | -1.64 | | | | | |
| AT3G20155 | (na) | VEAPS(ph)IGTAETK | -20.31 | | | | | |
| AT3G22150 | (na) | ELPIVIVNS(ph)LMVMY(ph)SR | -22.41 | | | | | |
| AT3G22430 | (na) | ES(ph)KPM(ox)M(ox)IGVQKT(ph)ADK | | | | -10.00 | | |
| AT3G23540 | (na) | AKFDIMELNT(ph)IK | 0.10 | | | | | |
| AT3G24750 | (na) | AVKFFT(ph)PKIM(ox)MELK | 27.84 | | | | | |
| AT3G25570 | (PAM) | IIIKT(ph)CGTTK | 29.58 | | | | | |
| AT3G25720 | (na) | RSSTS(ph)APSTLRK | -22.22 | | | | | |
| AT3G26240 | (na) | YS(ph)KPNMVDILNLFK | -0.67 | | | | | |
| AT3G27300 | (OPP) | QGS(ph)RGPAEADQLLK | -21.91 | | | | | |
| AT3G27380 | (TCA) | MAS(ph)GLIGRLVGT(ph)KPS(ph)K | -21.23 | | | | | |
| AT3G27470 | (na) | RPS(ph)QMMR | -20.94 | | | | | |
| AT3G27600 | (na) | ASYI(s)(t)GGLVVEKVMR | -20.97 | | | | | |
| AT3G27610 | (na) | FLVGGRNVDS(ph)VFK | -2.11 | 1.6400 | | -2.91 | | |
| AT3G29080 | (na) | RST(ph)IKVAYDEIFM(ox)ELSPSLK | -22.34 | | | -10.00 | | |
| AT3G29633 | (na) | (ac)KVPCDPLYT(ph)APR | | | | -10.00 | | |
| AT3G30845 | (na) | ET(ph)IM(ox)NMNMMTLK | -0.13 | | | | | |
| AT3G32050 | (na) | FKDVDFWIT(ph)MM(ox)QNK | 29.49 | | | | | |
| AT3G42800 | (na) | GS(ph)INENVSSSSSS(ph)PSPNK | 31.25 | | | | | |
| AT3G43890 | (na) | YETTQ(t)(s)LDLR | -19.70 | | | | | |
| AT3G43940 | (na) | YM(ox)LN(s)DGR(t)M(ox)GALSQEIGK | -23.43 | | | | | |
| AT3G44960 | (na) | S(ph)KS(ph)LGASTTNK | -1.46 | | | | | |
| AT3G46150 | (na) | (ac)MVT(ph)SSQVM(ox)LMPAHDSAK | | | | 3.22 | | |
| AT3G47910 | (na) | SQLS(ph)GTNGER | -19.72 | | | | | |
| AT3G48680 | (mit) | Y(ph)VTVGAYSLLR | 1.60 | | | | | |
| AT3G49290 | (na) | S(ph)AT(ph)FSFTSTIPKK | 27.45 | | | 10.00 | | |
| AT3G49290 | (na) | Y(ph)ILPAGEIMT(ph)ATNLEKLK | -1.04 | | | | | |
| AT3G50380 | (na) | VGY(ph)EVYADGLTRVIR | -21.16 | | | | | |
| AT3G50420 | (na) | T(ph)LLSACVNTR | -23.39 | | | | | |
| AT3G50740 | (sec) | T(ph)LKSLQDPK | -18.92 | | | | | |
| AT3G51760 | (na) | (ac)ILYLT(ph)VEEMM(ox)DINR | | | | -10.00 | | |
| AT3G53010 | (na) | AAM(ox)AS(ph)GGGGS(ph)YR | -1.79 | | | | | |
| AT3G53360 | (na) | VS(ph)VS(ph)NS(ph)QILAT(ph)SSVVST(ph)IK | 0.13 | | 3.35E-01 | -0.57 | 0.5252 | 3.35E-01 |
| AT3G53700 | (na) | S(ph)GS(ph)FDDMKKILEDMK | 1.26 | | | | | |
| AT3G55160 | (na) | Y(ph)VPLASLT(ph)RRLGAK | -22.16 | | | | | |
| AT3G56120 | (na) | M(ox)FDES(ph)KFDVNLK | 0.56 | | | | | |
| AT3G56360 | (na) | KRGGS(ph)VVSR | -22.63 | | | | | |
| AT3G56410 | (na) | SD(t)(s)(s)EKSILR | -3.45 | | | | | |
| AT3G56810 | (na) | RLEVS(ph)PPTTR | -21.12 | | | | | |
| AT3G56940 | (tet) | MS(ph)AS(ph)SSPPPPTTATSKSK | 10.60 | | | | | |
| AT3G57380 | (na) | IHDELTVE(s)(s)LMLGNK | -0.60 | | | 0.10 | | |
| AT3G57780 | (na) | QM(ox)VETLET(ph)RVEKLEEELR | 0.75 | | | | | |
| AT3G59120 | (na) | LSHTPHLPT(ph)K | -22.08 | | | | | |
| AT3G59260 | (na) | (ac)M(ox)KVMVLS(ph)LGKASPSK | -0.16 | | | -0.39 | | |
| AT3G60100 | (TCA) | ILPS(ph)AES(ph)GEEPLPESLLWLLLTGK | 0.81 | 0.1300 | 9.05E-01 | 0.71 | 0.8004 | |
| AT3G60920 | (na) | S(ph)LTSSKDNAAQKSSTKPK | -0.78 | 0.1400 | | -0.01 | | |
| AT3G61170 | (na) | M(ox)VEIY(ph)SKVDEM(ox)MLLIK | -1.36 | | | | | |
| AT3G61720 | (na) | LT(ph)EM(ox)T(ph)PLK | -14.65 | | | | | |
| AT3G63410 | (sec) | (ac)M(ox)AS(ph)LM(ox)LNGAITFPK | | | | 2.00 | | |
| AT4G01150 | (na) | ASSEETSSIDTNELITDLK | | | | -10.00 | | |
| AT4G01290 | (na) | QPNY(ph)GMPPPGS(ph)QVNR | -18.95 | 0.0465 | | | | |
| AT4G01290 | (na) | LSSISTPT(ph)EIQGYPIK | 4.27 | 0.0465 | | | | |
| AT4G01535 | (na) | LM(ox)LIES(ph)LNLLK | 29.26 | | | | | |
| AT4G02030 | (na) | M(ox)KDLIS(ph)GWIQK | 0.09 | | | | | |

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT4G02110 | (na) | SS(t)(s)PGSGLIR | -20.97 | | | | | |
| AT4G02110 | (na) | S(ph)IRRHSSLA(t)Y(s)R | -19.56 | | | | | |
| AT4G02550 | (na) | VKKES(ph)S(ph)HHLNDVK | -22.20 | | | | | |
| AT4G03070 | (sec) | Y(ph)STGLFSIPK | 2.46 | | | | | |
| AT4G03130 | (na) | SSPVSGAKGCQS(ph)LAK | -16.94 | | | | | |
| AT4G08320 | (na) | S(ph)GCQTFDVNSLAETLK | -0.41 | | | | | |
| AT4G10265 | (na) | (ac)MS(ph)SAS(ph)KTWM(ox)VAASIGAVEALK | | | | 10.00 | | |
| AT4G10465 | (na) | TTAHISY(ph)FRMSRK | -2.75 | | | | | |
| AT4G12900 | (na) | (ac)M(ox)ASSSSLASST(ph)K | | | | 1.61 | | |
| AT4G13030 | (na) | GGDTFKDDVVDEES(ph)SR | 0.16 | | | | | |
| AT4G13200 | (na) | NPDAVVVAS(ph)VT(ph)STSTVES(ph)K | -10.00 | | | -10.00 | | |
| AT4G14120 | (na) | YT(t)(s)QNLMASKLR | -20.69 | | | | | |
| AT4G14180 | (na) | LLNFLS(ph)LR | -17.44 | | | | | |
| AT4G16460 | (na) | S(ph)S(ph)T(ph)STGAESSNTGATDAK | 1.92 | | | | | |
| AT4G17000 | (na) | LEKNSAS(ph)RLK | 28.34 | | | | | |
| AT4G17250 | (na) | CLVT(ph)AVEVR | -1.04 | | | | | |
| AT4G17616 | (na) | M(ox)VLSGY(ph)YK | -24.52 | | | -10.00 | | |
| AT4G19350 | (na) | KSFKS(ph)TLR | 27.47 | | | | | |
| AT4G19460 | (na) | AWELY(ph)QEENKK | 0.94 | | | | | |
| AT4G21300 | (na) | MS(ph)ISSVAKR | -14.04 | | | | | |
| AT4G22190 | (na) | M(ox)S(ph)FGAPTTRK | -0.98 | | | | | |
| AT4G22430 | (na) | TCEKHMY(ph)KISR | 10.00 | | | 10.00 | | |
| AT4G22670 | (na) | SFVVEES(ph)DDDMDETEEVKPK | 1.30 | 0.1187 | | | | |
| AT4G23895 | (na) | S(ph)GSGFVSNSWIT(ph)GLLALPAAAFM(ox)LQDQEALAAEM(ox)ER | | | | 1.06 | | |
| AT4G24150 | (na) | HVES(ph)S(ph)HQS(ph)SHHNDIRT(ph)AKNDT(ph)S(ph)QLVR | | | | | | |
| AT4G24300 | (na) | FS(ph)RQVLSLLFPSK | 2.15 | | | | | |
| AT4G24320 | (na) | HQY(ph)IYLMSKSGRGK | -1.22 | | | | | |
| AT4G24490 | (sec) | S(ph)KDLN(s)LV(t)LDR | -21.64 | | | | | |
| AT4G24972 | (na) | M(ox)LLLS(ph)PGTGK | 0.70 | | | | | |
| AT4G25310 | (sec) | ERLS(ph)VASFHNTGFGK | 0.56 | | | | | |
| AT4G25770 | (na) | LYEQPGEVDSLDS(ph)PSK | | | | -10.00 | | |
| AT4G25845 | (na) | M(ox)VLS(ph)S(ph)ICLYPDK | | | | -0.90 | 0.5851 | |
| AT4G26630 | (na) | KT(ph)S(ph)PT(ph)AGSSS(ph)S(ph)K | | | | -10.00 | | |
| AT4G26965 | (mit) | FAGLFS(ph)S(ph)KSFIGVDK | -22.63 | | | | | |
| AT4G27040 | (na) | T(ph)DMMKEQLSTFR | -23.94 | | | | | |
| AT4G27585 | (na) | DHQETQALDETDLEELEDM(ox)GEK | | | | -0.98 | | |
| AT4G30790 | (na) | AS(ph)QYT(ph)ALRASAVK | -22.04 | | | | | |
| AT4G31080 | (na) | AAAATVLASKLGADS(ph)GLK | 4.25 | | | | | |
| AT4G31440 | (na) | S(ph)M(ox)NGTPGKHSLEK | -22.23 | | | | | |
| AT4G31520 | (na) | FYTYLQAY(ph)AK | 1.45 | | | | | |
| AT4G32130 | (na) | S(ph)QGVPS(ph)LTSLLPASR | -21.16 | | | | | |
| AT4G32285 | (na) | S(ph)FGDVNEIGAR | -21.39 | 0.2122 | | | | |
| AT4G32620 | (na) | LAVKIS(ph)GTTK | -18.87 | | | | | |
| AT4G34690 | (na) | NALYM(ox)HRLDEIT(ph)K | -1.48 | | | | | |
| AT4G35140 | (na) | S(ph)PVS(ph)KS(ph)ESSSS(ph)PK | 2.47 | 0.5290 | | 1.24 | 0.7496 | 2.24E-01 |
| AT4G36120 | (na) | FSIQEM(ox)QGS(ph)S(ph)T(ph)K | | | | 10.00 | | |
| AT4G36980 | (na) | QLT(ph)KQIK | 5.58 | | | | | |
| AT4G38260 | (na) | EGFQQLLAENGS(ph)GEFPVK | -0.38 | | | | | |
| AT4G38550 | (na) | EQIEDFYEQDDDVT(ph)PR | | | | 0.90 | | |
| AT4G38560 | (na) | T(ph)PLDKQS(ph)K | -14.73 | | | | | |
| AT4G39690 | (na) | SVLELS(ph)S(ph)RLSIK | 1.80 | | | | | |
| AT5G02060 | (na) | (ac)MKKM(ox)IGS(ph)PGTMSGLILR | 4.56 | | 2.66E-01 | 3.32 | 1.2925 | |
| AT5G02520 | (na) | NLKDNPAES(ph)REK | -21.17 | | | | | |
| AT5G03495 | (na) | M(ox)DES(ph)AIKGM(ox)K | -2.00 | | | | | |
| AT5G03660 | (na) | DQLSESM(ox)S(ph)FSSQM(ox)K | | | | -10.00 | | |
| AT5G03900 | (na) | MLTERCLS(ph)TR | -43.57 | | | | | |
| AT5G04750 | (mit) | (ac)M(ox)SSARS(ph)AITK | 1.32 | | 3.63E-01 | 10.00 | | |
| AT5G04810 | (na) | S(ph)IGS(ph)SSSSSSPSPIPSPKTPLK | -1.21 | | | | | |
| AT5G07630 | (na) | LGSLVVRM(ox)VFLPFEES(ph)SY(ph)TIFAR | -20.49 | | | | | |
| AT5G07630 | (na) | RDGPES(ph)EENVTRILK | 1.84 | | | | | |
| AT5G07830 | (na) | IMDPS(ph)Y(ph)LS(ph)QVSKTFK | 0.06 | | | | | |
| AT5G07940 | (na) | ETQS(ph)GHVGSRPSTSRK | -20.85 | | | | | |
| AT5G08050 | (na) | SGFSLS(ph)TIER | 0.95 | 0.1543 | | | | |
| AT5G08415 | (Co) | KS(ph)LMELEGK | -20.23 | | | | | |
| AT5G08415 | (Co) | TY(ph)GESIGFR | 0.89 | | | | | |
| AT5G08720 | (na) | IALMM(ox)NLS(ph)LAY(ph)K | | | | -10.00 | | |
| AT5G08770 | (na) | ELIS(ph)DTYRLATIGR | 10.37 | | | | | |
| AT5G10840 | (na) | VKVNKLT(ph)SIK | -0.28 | | | | | |
| AT5G11600 | (na) | AQTTST(ph)VVSLVK | 6.83 | | | | | |
| AT5G11660 | (na) | MKPS(ph)FSISKPK | 0.61 | | | | | |
| AT5G12930 | (na) | DS(ph)SGSHKR | -7.97 | | | | | |
| AT5G12930 | (na) | AVLEAPVDS(ph)VK | 2.63 | | | | | |
| AT5G14370 | (na) | KKEPDT(ph)T(ph)PFK | 4.83 | | | | | |
| AT5G14710 | (na) | M(ox)ES(ph)LDTNFPVRHRK | 1.50 | | | | | |
| AT5G18390 | (na) | Y(ph)ESM(ox)WKILK | 2.80 | | | | | |
| AT5G19050 | (na) | L(s)K(s)S(ph)S(ph)MAGGGSGSGDY(ph)GGPIK | 28.14 | | | | | |
| AT5G19820 | (na) | SIVDEIKQVMTAS(ph)SSR | -21.93 | | | | | |
| AT5G19950 | (na) | S(ph)LVT(ph)EISSLIGGGNK | 0.59 | | | | | |
| AT5G21005 | (na) | S(ph)MEKYIVGK | 27.88 | | | | | |
| AT5G21130 | (na) | QPAGNVT(ph)VIVTVLK | -1.95 | | | -3.90 | | |
| AT5G21970 | (na) | CLM(ox)MS(ph)VDKK | 3.20 | | | | | |
| AT5G22120 | (na) | ESEKT(ph)DESLK | 0.96 | | | | | |
| AT5G23430 | (na) | ETKSIGRSSTSQN(s)E(s)SMK | 31.22 | | | | | |
| AT5G24530 | (sec) | M(ox)KLYS(ph)DDPTKTTR | -10.73 | | | | | |
| AT5G25280 | (na) | RRSAY(ph)EPR | -2.38 | | | | | |
| AT5G25754 | (na) | EFNKILLY(ph)IFK | 4.93 | | | | | |
| AT5G25970 | (na) | KEY(ph)PFNRVPK | 29.08 | | | | | |
| AT5G26160 | (na) | VIIVS(ph)NVQAT(ph)RK | 3.60 | | | | | |
| AT5G27110 | (na) | (ac)M(ox)ESSKLLS(ph)LLR | 10.00 | | | 10.00 | | |
| AT5G27330 | (na) | VVALEKT(ph)NEAT(ph)GK | 4.52 | | | | | |
| AT5G28420 | (na) | QHPIAHGQYAFNAILS(ph)R | 0.03 | | | | | |
| AT5G28500 | (na) | (ac)MLSLTATTLS(ph)SSIFT(ph)QSK | | | | 1.22 | | |
| AT5G28730 | (na) | VLSAAIS(ph)DDPLFHVPPDS(ph)KYYLVDSGYANK | 28.87 | | | | | |
| AT5G28823 | (na) | S(ph)IQDYFLPAVS(ph)IDS(ph)AT(ph)TK | 0.68 | | | 0.44 | | |
| AT5G34940 | (na) | VIFGLNALS(ph)GRS(ph)IK | 0.00 | | | -0.88 | | |
| AT5G35300 | (na) | M(ox)ALILPY(ph)R | -0.54 | | 3.94E-01 | -0.77 | | 3.94E-01 |
| AT5G35405 | (na) | IGVPVS(ph)FE | 0.84 | | | | | |
| AT5G37960 | (na) | LATIIY(ph)KR | 29.73 | | | | | |

| | | | replicate 1 | | | replicate 2 | | |
|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2(ahk1/wt) | sd | p-value | log2(ahk1/wt) | sd | p-value |
| AT5G38220 | (na) | RGNEIVAIY(ph)IK | -0.78 | | | | | |
| AT5G40405 | (na) | MRET(ph)GLQVHGMTIR | -22.06 | | | | | |
| AT5G40450 | (na) | T(ph)ISVLDDSK | 1.42 | 0.6613 | | | | |
| AT5G40530 | (na) | (ac)TT(ph)EENKT(ph)S(ph)RNR | | | | 10.00 | | |
| AT5G41470 | (na) | AS(ph)M(ox)VNVT(ph)SIIVTRNLITK | -23.11 | | | | | |
| AT5G42680 | (na) | S(ph)SGSIGGGVLKMFK | -24.03 | | | | | |
| AT5G43160 | (na) | RLY(ph)NAWRSIS(ph)NLYNSVSMK | -10.00 | | | -10.00 | | |
| AT5G43211 | (na) | (ac)M(ox)ILYY(ph)M(ox)YCCGILVK | | | | 0.31 | | |
| AT5G47490 | (na) | LKSS(ph)G(s)VAG(s)R | -1.05 | | | | | |
| AT5G47890 | (mit) | (ac)M(ox)AWRGS(ph)ISKSMK | -0.22 | 0.2417 | | -0.45 | | |
| AT5G50510 | (na) | EALKILT(ph)DASK | -21.07 | | | | | |
| AT5G50940 | (na) | (ac)M(ox)SQSDSS(ph)PTKKQK | 0.96 | | 8.82E-01 | -1.52 | 6.3209 | 8.82E-01 |
| AT5G52680 | (na) | APAPT(ph)PKPAPAPPSVPAS(ph)VAAY(ph)ANM(ox)R | 32.42 | | | | | |
| AT5G53120 | (PAM) | (ac)MEGDVGIGLVCQNT(ph)MDGK | | | | -10.00 | | |
| AT5G53730 | (na) | M(ox)S(ph)QIS(ph)ITSPK | -1.98 | | | | | |
| AT5G53750 | (na) | S(ph)VS(ph)SAARM(ox)ARK | -0.42 | | | | | |
| AT5G54870 | (na) | VT(ph)WT(ph)WY(ph)FISR | | | | -10.00 | | |
| AT5G55210 | (na) | TS(s)RY(ph)(s)GPAAT(ph)AVFSGRVR | -20.74 | 0.1796 | | | | |
| AT5G55740 | (na) | (ac)M(ox)ASLPFNTIPNKVPFSVS(ph)SK | 0.71 | | | 0.48 | | |
| AT5G55820 | (na) | KFAPTWASKS(ph)NVR | 28.45 | | | | | |
| AT5G55840 | (na) | M(ox)Y(ph)DPARHILK | | | | 10.00 | | |
| AT5G57250 | (na) | FLRYLY(ph)R | -19.38 | | | | | |
| AT5G57590 | (sec) | RLGEFNRT(ph) | -20.67 | | | | | |
| AT5G58780 | (na) | TVQEIEEAT(ph)RSYKK | -21.25 | | | | | |
| AT5G58970 | (mit) | IPT(ph)GDGENLPK | -1.24 | | | | | |
| AT5G58990 | (na) | (ac)MAMT(ph)LM(ox)NRAISR | 1.06 | | | 1.23 | 0.4342 | 7.98E-02 |
| AT5G61020 | (na) | LGYQSAAYNAKGS(ph)Y(ph)GK | -20.49 | | | | | |
| AT5G61120 | (na) | FNING(t)PLLIF(s)SKLLDK | -23.28 | | | | | |
| AT5G64130 | (na) | AYFDS(ph)ADWALGK | -22.90 | | | | | |
| AT5G64190 | (na) | Y(ph)DVIKLVNEKLMS(ph)R | -13.32 | | | | | |
| AT5G64910 | (na) | KAY(ph)QEMRLYK | -21.44 | | | | | |
| AT5G65560 | (na) | TYTVLIKS(ph)LCGS(ph)ER | 1.94 | | | | | |
| AT5G65925 | (na) | SFT(ph)LNSATTTRER | -21.20 | | | | | |
| AT5G66200 | (na) | ENPNS(ph)T(ph)SATALPK | 4.78 | | | | | |
| AT5G67245 | (na) | T(ph)LRS(ph)FFSSLIKS | -1.38 | | | | | |
| ATMG00630 | (na) | (ac)MPSPILPM(ox)LPIS(ph)HLIGTEVR | | | | 4.19 | | |

## A34: Phosphopeptides which were quantified in *ahk1*-3 and Ws-2 in an experiment with a reciprocal metabolic labeling experimental design and 10min treatment with mock

Phosphopeptides (pPeptides) which were quantified in *ahk1-3* (ahk1) and the wildtype (wt) Ws-2 in an experiment with metabolic labeling after 10min treatment with mock, are listed in the tables of their respective functional category. The $log_2$-values of the ratio of normalized phosphopeptide ion intensities of *ahk1-3* and Ws-2 ($log_2$(ahk1/wt)) reveal more pPeptide abundance in *ahk1-3* ($log_2$>0) or in wt ($log_2$<0). sd gives the standard deviation, p-values show the respective statistical significance. Data were obtained from Waltraud X. Schulze.

### cell.organisation

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT4G14390 | FWIS(ph)NM(ox)LIINK | -0,7610 | 0,512 | 2,26E-01 |
| AT4G39050 | ATHIPY(ph)RDS(ph)K | -1,0968 | 0,302 | 1,79E-02 |

### cell.vesicle transport

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT3G11820 | (ac)M(ox)NDLFSSSFS(ph)R | NaN | | 1,00E+00 |
| AT3G11820 | ASS(ph)FIRGGTDQLQTAR | 0,8621 | 0,645 | 2,28E-01 |
| AT4G12770 | FENVFSSIS(ph)SSPTK | -0,4244 | 0,281 | 7,51E-01 |
| AT4G12770 | FENVFSSISS(ph)SPTK | NaN | | 1,00E+00 |
| AT4G12770 | FENVFSSISSS(ph)PTK | 0,6893 | 0,262 | 1,33E-01 |

### development.

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT1G15750 | APS(ph)PVNNPLLGGIPK | 0,1466 | 0,043 | 7,17E-01 |
| AT1G80490 | APS(ph)PVNNPLLGSLPK | -0,1908 | 0,081 | 5,79E-01 |
| AT3G18390 | GFALIY(ph)YR | NaN | | 1,00E+00 |
| AT3G48740 | LGTVSS(ph)PEPISVVR | -0,4667 | 0,212 | 9,69E-01 |

### protein.degradation

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT1G66670 | VMIHQPLGT(ph)AGGK | NaN | | 1,00E+00 |
| AT3G51800 | (ac)S(ph)SDDERDEKELSLTSPEVVTK | -0,6569 | 0,191 | 1,65E-01 |

### protein.posttranslational modification

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT1G12310 | SIIASENLS(ph)SPFDFNRFLDLMAK | NaN | | 1,00E+00 |
| AT1G22280 | TDQAILSNS(ph)SDLGR | 0,6573 | 0,499 | 2,43E-01 |
| AT2G17700 | VQIESGVMT(ph)AETGTYR | -2,2735 | 1,722 | 1,82E-01 |

### RNA.regulation of transcription

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT2G02070 | TPNSDAEVIALS(ph)PK | NaN | | 1,00E+00 |
| AT2G41900 | NVVEPIS(ph)PMSAR | -0,3590 | 0,115 | 5,71E-01 |
| AT3G61260 | IALESES(ph)PAK | 0,4002 | #DIV/0! | 1,00E+00 |
| AT4G22140 | LSLFS(ph)HLLY(ph)R | -0,2982 | #DIV/0! | 1,00E+00 |

### signaling.

(Ca) calcium; (G) G-proteins; (RK) receptor kinases; (14) 14-3-3 proteins

| accession | category | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|---|
| AT1G06840 | (RK) | LAPVPDMEGIS(ph)PQHVSTVVK | -0,3949 | 0,181 | 2,75E-01 |
| AT1G34300 | (RK) | ISEGS(ph)M(ox)LGS | 0,1091 | 0,098 | 6,50E-01 |
| AT1G34300 | (RK) | ISEGSM(ox)LGS(ph) | NaN | | 1,00E+00 |
| AT1G53430 | (RK) | LNDDENTHIS(ph)TR | 0,5312 | 0,322 | 6,28E-01 |
| AT2G43130 | (G) | (ac)S(ph)DDDERGEEYLFK | 0,7111 | 0,331 | 3,15E-01 |
| AT3G02880 | (RK) | LIEEVSHSSGS(ph)PNPVSD | -1,2614 | 0,694 | 1,35E-01 |
| AT3G14840 | (RK) | LDEEENTHIS(ph)TR | 0,6910 | #DIV/0! | 1,00E+00 |
| AT4G13350 | (G) | DLGSAS(ph)PPVARPVR | -0,1432 | 0,042 | 9,79E-01 |
| AT4G29900 | (Ca) | (ac)SGQFNNS(ph)PRGEDK | -0,2562 | #DIV/0! | 1,00E+00 |
| AT4G33050 | (Ca) | SLS(ph)FNSWEVPK | 0,6609 | #DIV/0! | 1,00E+00 |
| AT4G35310 | (Ca) | NSLNIS(ph)MRDA | 0,2415 | 0,031 | 3,62E-01 |
| AT5G16050 | (14) | (ac)S(ph)SDSSREENVYLAK | -0,4345 | 0,084 | 1,67E-01 |
| AT5G16590 | (RK) | LIEEVSRSPAS(ph)PGPLSD | 0,2222 | 0,054 | 1,71E-01 |
| AT5G20480 | (RK) | TLANIS(ph)SLER | NaN | | 1,00E+00 |

### stress.

(a) abiotic; (b) biotic

| accession | category | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|---|
| AT1G11310 | (b) | SVENYPSSPS(ph)PR | -0,0031 | 0,001 | 8,86E-01 |
| AT5G56030 | (a) | EIS(ph)DDEEEEEK | -0,2300 | #DIV/0! | 1,00E+00 |
| AT5G56030 | (a) | EIS(ph)DDEEEEEKKDEEGK | -1,2245 | #DIV/0! | 1,00E+00 |

## transport.

(ABC) ABC transorters; (am) ammonium; (ATP) p- and v-ATPases; (Ca) calcium; (Aq) Major Intrinsic Proteins; (K) potassium; (su) sugars

| accession | category | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|---|
| AT1G75220 | (su) | (ac)S(ph)FRDDNEEAR | 0,1006 | 0,027 | 4,11E-01 |
| AT2G18960 | (ATP) | GLDIDTAGHHYT(ph)V | -0,4294 | 0,225 | 3,62E-01 |
| AT3G53420 | (Aq) | SLGS(ph)FRS(ph)AANV | 0,2657 | 0,141 | 5,16E-01 |
| AT3G53420 | (Aq) | SLGS(ph)FRSAANV | 0,1187 | 0,040 | 8,24E-01 |
| AT3G53420 | (Aq) | SLGSFRS(ph)AANV | -0,5718 | 0,383 | 9,90E-01 |
| AT3G58730 | (ATP) | GIS(ph)INAAR | NaN | | 1,00E+00 |
| AT3G62700 | (ABC) | SIS(ph)IESPRQPK | 1,0170 | 0,698 | 2,47E-01 |
| AT4G13510 | (am) | ISSEDEMAGMDMT(ph)R | -0,5465 | 0,245 | 3,75E-01 |
| AT4G23640 | (K) | SIS(ph)EANIAGSSR | 0,8788 | 0,473 | 2,89E-01 |
| AT4G30190 | (ATP) | GLDIETPSHYT(ph)V | -0,5369 | 0,231 | 2,50E-01 |
| AT4G30190 | (ATP) | LKGLDIETPSHYT(ph)V | -0,6039 | 0,154 | 3,68E-01 |
| AT4G35100 | (Aq) | ALGS(ph)FRS(ph)NATN | 0,1348 | 0,043 | 9,71E-01 |
| AT4G35100 | (Aq) | ALGS(ph)FRSNATN | 0,1965 | 0,088 | 9,87E-01 |
| AT4G35100 | (Aq) | ALGSFRS(ph)NATN | 0,4956 | 0,218 | 4,79E-01 |
| AT5G17010 | (su) | SSGEIS(ph)PEREPLIK | 0,1720 | 0,125 | 6,30E-01 |
| AT5G57110 | (Ca) | (ac)TSLLKS(ph)SPGR | 0,0588 | 0,011 | 3,85E-01 |
| AT5G57110 | (Ca) | (ac)TSLLKS(ph)SPGRR | 0,3949 | #DIV/0! | 3,85E-01 |
| AT5G57110 | (Ca) | (ac)TSLLKSS(ph)PGR | 0,0380 | 0,005 | 8,37E-01 |
| AT5G60660 | (Aq) | ALGSFGS(ph)FGSFR | 0,1400 | 0,068 | 2,68E-01 |
| AT5G60660 | (Aq) | ALGSFGSFGS(ph)FR | NaN | | 2,68E-01 |

## others - cell wall.

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT1G53840 | (ac)MDSVNS(ph)FKGYGK | -0,1986 | 0,049 | 5,63E-01 |
| AT1G65570 | SIEEQGVENVT(ph)VK | NaN | | 1,00E+00 |
| AT3G29360 | DLSMNKFDWDHPLHLQPMS(ph)PTTVK | 0,2978 | 0,109 | 6,64E-01 |
| AT3G29360 | FDWDHPLHLQPMS(ph)PTTVK | 0,8033 | 0,301 | 8,30E-02 |
| AT3G51160 | (ac)ASENNGS(ph)RSDSESITAPK | -0,4611 | 0,222 | 2,43E-01 |
| AT3G51160 | (ac)ASENNGSRS(ph)DSESITAPK | NaN | | 2,43E-01 |

## others - cell.division

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT2G21280 | ALLGEGAT(ph)VVLEGQK | -0,0254 | 0,007 | 4,41E-01 |

## others - DNA.unspecified

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT3G42170 | (ac)MEVYNDDTEMRS(ph)PETQPIK | 0,4053 | #DIV/0! | 1,00E+00 |

## others - gluconeogenesis / glyoxylate cycle.

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT4G15530 | GGM(ox)T(ph)SHAAVVAR | 0,3066 | 0,158 | 8,02E-01 |
| AT4G15530 | GGMT(ph)SHAAVVAR | -0,0510 | 0,042 | 9,57E-01 |

## others - glycolysis.

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT1G07110 | SLS(ph)ASSFLIDTK | 0,8413 | 0,648 | 2,02E-01 |
| AT1G53310 | M(ox)AS(ph)IDVHLR | 0,1581 | 0,088 | 4,01E-01 |
| AT1G53310 | MAS(ph)IDVHLR | 0,3080 | 0,264 | 7,20E-01 |
| AT1G70730 | ATGAFILTAS(ph)HNPGGPTEDFGIK | 0,6248 | 0,341 | 2,29E-01 |
| AT2G42600 | M(ox)AS(ph)IDAQLR | 0,2528 | 0,106 | 7,51E-01 |
| AT2G42600 | MAS(ph)IDAQLR | -0,2353 | 0,120 | 8,01E-01 |
| AT3G52930 | LGDGAAES(ph)LHVK | -2,1329 | 1,134 | 4,40E-02 |

## others - hormone metabolism.

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT2G34680 | PVIS(ph)SNLIK | NaN | | 1,00E+00 |
| AT2G34680 | PVISSNLIK | NaN | | 1,00E+00 |
| AT4G27450 | RGS(ph)EANWSL | 0,6969 | 0,412 | 1,07E-01 |
| AT4G27450 | VDVYNRVNS(ph)IPR | -0,5122 | 0,335 | 3,44E-01 |
| AT5G43830 | VDS(ph)SQNWAGHI | 0,0545 | 0,025 | 8,15E-01 |

## others - lipid metabolism.

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT3G05630 | (ac)S(ph)TDKLLLPNGVK | -0,9186 | 0,336 | 3,79E-02 |
| AT5G35360 | LLEEAPSPALT(ph)AELRK | NaN | | 1,00E+00 |

## others - major CHO metabolism.

(d) degradation; (s) synthesis

| accession | category | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|---|
| AT1G35580 | (d) | SVLDT(ph)PLSSAR | -0,1529 | 0,113 | 7,23E-01 |
| AT1G74910 | (s) | RVS(ph)SFEALQPATR | -0,2181 | 0,134 | 9,17E-01 |
| AT1G74910 | (s) | VSS(ph)FEALQPATR | NaN | | 1,00E+00 |
| AT4G34860 | (d) | SLTELTGS(ph)PQLR | -0,6762 | 0,210 | 3,23E-01 |
| AT5G20280 | (s) | INS(ph)AESMELWASQQK | -0,9032 | 0,375 | 1,36E-01 |

## others - minor CHO metabolism.

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT5G40390 | SDS(ph)GINGVDFTEK | 0,3959 | 0,180 | 3,07E-01 |

## others - misc.

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT1G16610 | VSS(ph)PPKPVSAAPK | 1,3996 | 0,941 | 7,21E-02 |
| AT1G28030 | T(ph)M(ox)TLESFGLEK | 0,5867 | 0,286 | 4,66E-01 |
| AT2G30860 | VYGPHFAS(ph)PK | -0,1379 | 0,042 | 5,62E-01 |
| AT3G45190 | IPNGSSSSEGEIS(ph)PR | -1,3124 | 0,570 | 6,22E-02 |
| AT4G39480 | DTILS(ph)FM(ox)LAGR | NaN | | 1,00E+00 |

## others - N-metabolism.

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT1G37130 | SVS(ph)TPFM(ox)NTTAK | 0,3633 | 0,114 | 1,77E-01 |
| AT1G37130 | SVS(ph)TPFMNTTAK | 0,7208 | 0,405 | 5,13E-01 |

## others - protein.

(a) amino acid activation; (f) folding; (g) glycosylation; (s) synthesis; (t) targeting

| accession | category | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|---|
| AT1G26630 | (s) | (ac)S(ph)DDEHHFEASESGASK | -0,1368 | 0,073 | 7,54E-01 |
| AT1G64790 | (s) | ALLEGGS(ph)DDEGASTEAQGR | -0,0186 | 0,009 | 9,84E-01 |
| AT1G72160 | (t) | SMIPQNLGS(ph)FKEESSK | -0,1296 | 0,047 | 6,94E-01 |
| AT2G41840 | (s) | ALS(ph)TSKPDPVVEDQA | -0,8714 | 0,541 | 7,52E-01 |
| AT2G41840 | (s) | ALST(ph)SKPDPVVEDQA | NaN | | 1,00E+00 |
| AT2G41840 | (s) | ALSTS(ph)KPDPVVEDQA | 0,8761 | 0,332 | 1,67E-01 |
| AT3G57150 | (s) | EEVIEEVAS(ph)PK | NaN | | 1,00E+00 |
| AT4G02510 | (t) | VGADDLS(ph)DSEK | 0,5564 | 0,428 | 6,09E-01 |
| AT4G31700 | (s) | S(ph)RLSSAAAKPSVTA | -0,1812 | 0,056 | 5,25E-01 |
| AT4G31700 | (s) | SRLS(ph)SAAAKPSVTA | NaN | | 1,00E+00 |
| AT5G27640 | (s) | DGEVS(ph)DVEEDEYEAK | 0,7354 | 0,727 | 2,54E-01 |

## others - photosynthesis

(CC) calvin cycle; (LR) light reaction

| accession | category | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|---|
| AT2G46820 | (LR) | AT(ph)TEVGEAPATTTEAETTELPEIVK | NaN | | 1,00E+00 |
| ATCG00020 | (LR) | (ac)T(ph)AILERR | 0,8359 | 0,586 | 5,15E-01 |
| ATCG00270 | (LR) | (ac)T(ph)IALGKFTK | -0,1778 | 0,145 | 7,42E-01 |

## others - redox.

| accession | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|
| AT1G21750 | SADDASEVVS(ph)DKK | -0,3846 | 0,077 | 1,17E-01 |
| AT3G08710 | VTSIIDSVPES(ph)PQRP | -0,5491 | 0,346 | 7,10E-01 |

## others - RNA

(p) processing; (t) transcription

| accession | category | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|---|
| AT2G29210 | (p) | LPS(ph)PSIEQR | -0,1549 | 0,073 | 2,63E-01 |
| AT3G26560 | (p) | YSVDMS(ph)PVK | 0,1557 | 0,095 | 9,01E-01 |
| AT3G46780 | (t) | SQPLT(ph)ISDLIEK | -0,4046 | 0,255 | 9,83E-01 |
| AT3G46780 | (t) | SQPLTIS(ph)DLIEK | NaN | | 1,00E+00 |
| AT3G57660 | (t) | GVM(ox)NDLLS(ph)DGLLK | -0,2418 | 0,084 | 8,24E-01 |
| AT5G52040 | (p) | ERVAS(ph)PENGAVR | 0,1653 | 0,069 | 9,47E-01 |

## others

(f) fermentation; (OPP) OPP; (TCA) TCA/org transformation; (na) not assigned

| accession | category | pPeptide | log2 (ahk1/wt) | sd | p-value |
|---|---|---|---|---|---|
| AT1G59900 | (TCA) | YHGHS(ph)MSDPGSTYR | 0.3567 | 0.268 | 4.17E-01 |
| AT1G70770 | (na) | M(ox)TAIDS(ph)DDDGVVR | 0.9766 | 0.684 | 3.25E-01 |
| AT1G70770 | (na) | MTAIDS(ph)DDDGVVR | 0.4331 | 0.149 | 3.00E-01 |
| AT1G76850 | (na) | VALTSLQS(ph)LPR | -0.2625 | 0.111 | 5.53E-01 |
| AT1G77120 | (f) | IIGVDFNS(ph)K | 1.1782 | 0.728 | 2.24E-01 |
| AT2G20010 | (na) | VQMRIS(ph)EQIDSRIR | 0.3800 | 0.340 | 9.21E-01 |
| AT2G37970 | (na) | IEMTS(ph)PVVTK | 0.5034 | | 1.00E+00 |
| AT2G46980 | (na) | LSQDKGS(ph)NDDPLIK | NaN | | 1.00E+00 |
| AT3G05900 | (na) | TSESGSALS(ph)PEK | -0.2015 | 0.111 | 6.87E-01 |
| AT3G09560 | (na) | FYDFQDDPPS(ph)PTSEYGSAR | NaN | | 1.00E+00 |
| AT3G50620 | (na) | DIVEY(ph)FNRR | -1.1647 | 0.322 | 4.42E-02 |
| AT3G63000 | (na) | GGPAVTPAGS(ph)FGR | NaN | | 1.00E+00 |
| AT4G04790 | (na) | IYM(ox)SLIDAY(ph)AAS(ph)GK | -0.5827 | 0.196 | 3.75E-01 |
| AT4G15802 | (na) | AEMGVEGT(ph)PPPASK | 0.4648 | 0.342 | 4.12E-01 |
| AT4G16144 | (na) | NVVDLY(ph)IM(ox)LLR | NaN | | 1.00E+00 |
| AT4G20220 | (na) | ENVSEAKLVVGM(ox)T(ph)IR | -0.6395 | 0.223 | 2.22E-01 |
| AT4G32285 | (na) | S(ph)FGDVNEIGAR | 0.5229 | 0.366 | 8.83E-01 |
| AT5G07760 | (na) | TVLSLY(ph)S(ph)VVGK | NaN | | 1.00E+00 |
| AT5G35200 | (na) | VVEEKPAS(ph)PEPVK | NaN | | 1.00E+00 |
| AT5G40450 | (na) | S(ph)LSDHIQKEPK | -0.2503 | 0.087 | 2.82E-01 |
| AT5G40760 | (OPP) | STFRNDS(ph)FVR | 1.0418 | 0.646 | 1.64E-01 |
| AT5G52290 | (na) | TEEM(ox)ILHS(ph)VR | NaN | | 1.00E+00 |
| AT5G53420 | (na) | LGAGLVQS(ph)PLDR | NaN | | 1.00E+00 |
| AT5G64430 | (na) | FSYNSYPDSTDS(ph)SPR | -0.6770 | 0.410 | 3.54E-01 |

## A35: Phosphopeptides which were quantified in ahk1-3 and Ws-2 in an experiment without metabolic labeling after 10min treatment with 0.3M mannitol or mock

Phosphopeptides (pPeptides) which were quantified in *ahk1-3* (ahk1) and the wildtype (wt) Ws-2 in an experiment without metabolic labeling after 10min treatment with 0.3M mannitol (ahk1.m; wt.m) or mock (ahk1; wt), are listed in the tables of their respective functional category. The $log_2$-values of the ratio of normalized phosphopeptide ion intensities reveal more pPeptide abundance in *ahk1-3* ($log_2$>0) or in wt ($log_2$<0). p-values show the respective statistical significance. Data were obtained from Waltraud X. Schulze.

cell.organisation

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G04780 | FEELEAIEDEFVT(ph)PPS(ph)SPTSSVK | -1.95 | 1.11E-01 | | 1.00E+00 | | 1.00E+00 | 0.55 | 3.23E-01 |
| AT1G04780 | LDS(ph)PEESSNGESSR | 0.81 | 8.77E-02 | 0.66 | 1.35E-01 | -0.12 | 6.15E-01 | -0.27 | 5.49E-01 |
| AT1G04780 | QLELALKLDS(ph)PEESSNGESSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G04780 | VSDLLGDDDS(ph)PSR | 0.30 | 4.58E-01 | 0.54 | 1.61E-01 | -0.30 | 4.38E-01 | -0.06 | 9.07E-01 |
| AT1G04780 | VSDLLGDDDS(ph)PSRGGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G24460 | EALTT(ph)DDDDNDDLGTHFNIEK | -0.51 | 1.63E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G42550 | GVEFDDDLETEKS(ph)DGT(ph)IGER | -1.25 | 3.77E-02 | -0.01 | 9.92E-01 | -0.61 | 1.64E-01 | 0.64 | 4.05E-01 |
| AT1G42550 | NFANS(ph)FGR | | 1.00E+00 | 0.39 | 4.99E-01 | -1.39 | 8.36E-02 | | 1.00E+00 |
| AT1G42550 | SGES(ph)VDDESENYLSDLGK | -0.77 | 1.47E-01 | -0.29 | 4.67E-01 | -0.18 | 7.64E-01 | 0.31 | 6.00E-01 |
| AT1G42550 | TSFSVPS(ph)PK | 0.45 | 1.98E-01 | 0.52 | 9.90E-01 | -0.82 | 6.97E-01 | -0.75 | 1.22E-03 |
| AT1G47200 | (ac)AETAETINTT(ph)ISSPPPESESSTTISAMTDPTSQEAASK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G52080 | DLSTTLS(ph)PTSEEK | | 1.00E+00 | 1.74 | 5.46E-02 | -0.43 | 4.48E-01 | | 1.00E+00 |
| AT1G63640 | LVGPTS(ph)PR | | 1.00E+00 | 0.03 | 8.48E-01 | -0.88 | 4.40E-02 | | 1.00E+00 |
| AT2G31820 | QVS(ph)FQGVNVENYQQSR | | 1.00E+00 | -0.30 | 2.61E-01 | 0.01 | 4.85E-01 | | 1.00E+00 |
| AT2G37080 | TGS(ph)LES(ph)PLR | | 1.00E+00 | 0.92 | 3.11E-02 | | 1.00E+00 | -0.40 | 1.48E-01 |
| AT2G38750 | S(ph)FFVEDEER | 0.14 | 7.55E-01 | 0.98 | 5.19E-02 | -0.36 | 5.46E-01 | 0.48 | 2.88E-01 |
| AT2G41740 | AAAVAALSQVLVAENKKS(ph)PDT(ph)SPTR | -0.57 | 6.58E-01 | | 1.00E+00 | | 1.00E+00 | 0.29 | 4.99E-01 |
| AT2G41740 | ST(ph)SS(ph)NPADDIPLTEAK | -0.20 | 5.68E-01 | 0.73 | 8.33E-02 | -0.28 | 4.74E-01 | 0.65 | 6.94E-02 |
| AT2G41740 | ST(ph)SS(ph)NPADDIPLTEAKDEEEASEVAGLEAK | -1.78 | 5.16E-02 | | 1.00E+00 | 1.36 | 1.39E-01 | | 1.00E+00 |
| AT3G57410 | AEALAALTSAFNS(ph)SPSSKSPPR | | 4.28E-01 | | 1.00E+00 | | 1.00E+00 | | 4.52E-01 |
| AT3G57410 | AEALAALTSAFNSSPS(ph)SKSPPRR | | 4.28E-01 | | 1.00E+00 | | 1.00E+00 | | 4.52E-01 |
| AT3G57410 | AEALAALTSAFNSSPSS(ph)KSPPRR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G57410 | KKS(ph)PDTSPSAEAK | 1.39 | 2.26E-01 | 0.74 | 7.87E-01 | -0.02 | 7.00E-01 | -0.67 | 4.97E-01 |
| AT3G57410 | SGLTSQAS(ph)QR | 0.18 | 2.86E-01 | 0.25 | 6.18E-01 | -0.96 | 1.32E-01 | -0.90 | 3.01E-01 |
| AT3G57890 | (ac)TEEQDLIDQS(ph)PPPSAPDPEPNSSTNPNSVIHPR | -0.36 | 9.66E-01 | | 1.00E+00 | | 1.00E+00 | 0.75 | 7.67E-01 |
| AT4G16340 | GPVSEGAGS(ph)PK | -0.97 | 2.02E-01 | 0.45 | 7.84E-01 | -0.35 | 9.26E-01 | 1.07 | 1.59E-01 |
| AT4G16340 | LEGT(ph)PDNGYLWQR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G16340 | S(ph)ATIEEDVASISGRPFSDPGSSK | -2.75 | 2.83E-02 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G16340 | VNSQLAS(ph)PSQPYSLR | | 1.00E+00 | 0.48 | 3.86E-02 | -0.68 | 9.79E-03 | | 1.00E+00 |
| AT4G26760 | ADPVMAS(ph)P | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G27430 | DNDS(ph)EPEELLR | 0.26 | 4.63E-01 | 0.50 | 1.42E-01 | 0.03 | 9.24E-01 | 0.27 | 3.53E-01 |
| AT4G30160 | GRS(ph)PAFNALAATFESQNAR | -2.10 | 1.58E-01 | -2.31 | 1.42E-02 | 1.83 | 1.85E-02 | 1.63 | 2.90E-01 |
| AT4G30160 | NLS(ph)TPPPVVR | 0.57 | 1.63E-01 | 0.17 | 6.44E-01 | -1.03 | 6.95E-02 | -1.42 | 2.48E-02 |
| AT4G30160 | S(ph)PAFNALAATFESQNAR | -0.95 | 1.78E-01 | -1.59 | 4.49E-02 | 1.20 | 2.10E-01 | 0.56 | 5.70E-01 |
| AT4G30160 | SM(ox)SFS(ph)PDRVR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G33200 | TLITS(ph)PER | 0.08 | 9.65E-01 | | 1.00E+00 | | 1.00E+00 | -0.17 | 3.30E-01 |
| AT5G10470 | SDAALLNLEEGS(ph)SPIPNPSTAAEDSR | -0.48 | 3.87E-01 | -0.09 | 4.76E-01 | 0.07 | 2.79E-01 | 0.45 | 2.90E-01 |
| AT5G20490 | ENS(ph)GFGFLLTR | 0.11 | 5.25E-01 | -0.50 | 7.17E-02 | 1.17 | 1.17E-03 | 0.56 | 6.14E-02 |
| AT5G20490 | GSPQSAGLS(ph)FLNR | -0.76 | 2.12E-01 | 0.33 | 5.18E-01 | -0.70 | 2.20E-01 | 0.38 | 2.70E-01 |
| AT5G20490 | QQALAIS(ph)PTSR | 0.10 | 2.72E-01 | | 1.00E+00 | 0.06 | 7.83E-01 | | 1.00E+00 |
| AT5G55230 | EEAASSPVSGAADHQVPAS(ph)P | | 1.00E+00 | 1.69 | 1.00E+00 | -0.70 | 1.85E-01 | | 1.00E+00 |
| AT5G65460 | SDAALFTLEEGS(ph)SPVQNPSTAAEDSR | -0.12 | 8.13E-01 | 0.05 | 3.64E-01 | 0.33 | 3.37E-02 | 0.50 | 1.74E-01 |
| AT5G65460 | SSSGSS(ph)SPGRSPVR | | 1.00E+00 | | 3.64E-01 | | 3.37E-02 | | 1.00E+00 |
| AT5G65460 | SVASSTQVSS(ph)PSSK | 0.29 | 6.42E-01 | -0.13 | 7.63E-01 | 0.24 | 5.59E-01 | -0.18 | 8.68E-01 |

cell.vesicle transport

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G06210 | IELGLS(ph)S(ph)DEDEK | 0.41 | 9.03E-02 | 0.84 | 6.03E-03 | 0.00 | 9.71E-01 | 0.42 | 1.67E-01 |
| AT1G08190 | EDNNRSS(ph)FSQR | 0.47 | 2.05E-02 | 0.19 | 4.08E-01 | -0.58 | 2.45E-02 | -0.86 | 4.83E-03 |
| AT1G08190 | REDNNRS(ph)SFSQR | | 2.05E-02 | | 1.00E+00 | | 1.00E+00 | | 4.83E-03 |
| AT1G08820 | VTLVPPS(ph)DSPELSPINTPK | 0.25 | 4.05E-01 | -0.16 | 6.14E-01 | 0.81 | 5.96E-02 | 0.40 | 4.53E-01 |
| AT1G12360 | GS(ph)DDGYSS(ph)DSVLR | | 1.00E+00 | -1.23 | 9.76E-02 | 1.32 | 5.96E-02 | | 1.00E+00 |
| AT1G12360 | GS(ph)DDGYSSDSVLR | -0.11 | 8.37E-01 | | 1.00E+00 | | 1.00E+00 | 0.72 | 2.16E-01 |
| AT1G64180 | SQS(ph)PSSLQVK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G76970 | APPPVQIVDINHDDEDDES(ph)DDEFAR | 0.05 | 6.04E-01 | -0.38 | 6.84E-02 | 1.03 | 8.77E-03 | 0.61 | 3.31E-01 |
| AT2G20790 | KGDGDDEES(ph)EDESAENVVNVEDFLVQK | | 1.00E+00 | | 5.83E-02 | | 7.91E-02 | 1.46 | 1.00E+00 |
| AT2G20790 | RKGDGDDEES(ph)EDESAENVVNVEDFLVQK | | 1.00E+00 | | 5.83E-02 | | 7.91E-02 | | 1.00E+00 |
| AT2G38410 | DSSSIAGSSS(ph)PIPATVSTGK | 0.29 | 3.88E-01 | 0.99 | 7.10E-02 | -0.71 | 3.06E-01 | -0.01 | 9.67E-01 |
| AT2G38410 | HDAIASGSPLPVQASGS(ph)PLSVQASKPADSSPK | -2.72 | 4.71E-04 | | 1.00E+00 | | 1.00E+00 | 2.81 | 4.64E-02 |
| AT2G38410 | RS(ph)PDASPIITPPVSHPPLR | | 4.71E-04 | | 1.00E+00 | | 1.00E+00 | | 4.64E-02 |
| AT2G38410 | SLQQSNS(ph)FPTR | -0.47 | 5.41E-01 | 0.24 | 6.15E-01 | -0.53 | 3.51E-01 | 0.18 | 8.81E-01 |
| AT2G45200 | ASGSM(ox)S(ph)PGVQVLR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G45200 | FTQGGYVDTGS(ph)PTVGSGR | 0.48 | 2.00E-01 | 0.64 | 1.91E-02 | -0.41 | 1.02E-01 | -0.25 | 5.04E-01 |
| AT3G10380 | ASQHDINT(ph)PR | 2.25 | 7.37E-02 | | 1.00E+00 | | 1.00E+00 | -2.14 | 5.65E-02 |
| AT3G11820 | (ac)M(ox)NDLFSSS(ph)FSR | 0.19 | 6.39E-01 | 1.17 | 3.71E-03 | -0.41 | 1.69E-01 | 0.57 | 9.12E-02 |
| AT3G11820 | LIS(ph)TGESER | 0.59 | 1.55E-01 | 1.33 | 3.66E-02 | -1.18 | 4.74E-02 | -0.43 | 4.63E-02 |
| AT3G14090 | S(ph)TSSIREM(ox)DLISPEAVSDLR | -2.24 | 1.13E-01 | | 1.00E+00 | | 1.00E+00 | 1.40 | 1.00E+00 |
| AT3G17440 | ELKDEEARNS(ph)PEVNK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -3.89 | 1.02E-02 |
| AT3G52400 | (ac)MNDLLSGS(ph)FK | 1.10 | 1.95E-04 | 1.10 | 2.23E-04 | 0.11 | 4.44E-01 | 0.11 | 5.18E-01 |

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT3G52400 | TSVADGSS(ph)PPHSHNIEM(ox)SK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G08180 | DFLSSS(ph)SFK | 1.65 | 1.00E+00 | -0.03 | 4.80E-01 | 0.69 | 1.00E+00 | -0.99 | 1.63E-02 |
| AT4G11380 | TEDEDFAEGSEAGYSSSNPVDSAAS(ph)PPGNIPQPSGR | -0.63 | 1.50E-01 | 0.29 | 1.48E-01 | 0.17 | 1.41E-01 | 1.10 | 2.69E-02 |
| AT4G11740 | EAPVNDDDEM(ox)DIDDVIPAPQS(ph)PLSM(ox)FNAAR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G11740 | LAAPSSPFDDDS(ph)DDVDEQPLVR | -0.71 | 2.61E-01 | -0.14 | 8.31E-01 | 0.48 | 4.94E-01 | 1.05 | 1.18E-01 |
| AT4G12770 | AS(ph)VNSPTASQM(ox)DELDDFSIGR | -0.24 | 6.73E-01 | -0.02 | 1.00E+00 | -0.25 | 4.39E-02 | -0.03 | 3.99E-01 |
| AT4G12770 | FENVFSSISSS(ph)PTK | 0.91 | 8.13E-02 | 0.35 | 6.41E-01 | 0.39 | 6.03E-01 | -0.17 | 3.61E-01 |
| AT4G12770 | QQQENNNDLDSFFNSVSRPS(ph)SVPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G12780 | QQQENTNDLDS(ph)FFSSISRPNSAPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G12780 | QQQENTNDLDSFFSSISRPNS(ph)APR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G21450 | YLAQQQGEGADS(ph)V | 0.35 | 2.76E-01 | 0.61 | 5.44E-02 | -0.52 | 1.12E-01 | -0.25 | 4.81E-01 |
| AT4G23460 | TEDEDYVEGSETGYPEASGNPVDGAAS(ph)PSATTGYVTK | -0.35 | 1.47E-01 | -0.23 | 1.00E+00 | 0.39 | 1.00E+00 | 0.52 | 1.03E-01 |
| AT5G03540 | ILQSSSAQGLTS(ph)SGGGSLEGGNSSGVSR | -0.05 | 8.09E-01 | | 1.00E+00 | | 1.00E+00 | 0.09 | 6.88E-01 |
| AT5G08080 | (ac)M(ox)NDLLKGS(ph)FELPR | -0.31 | 4.79E-01 | -0.78 | 2.93E-01 | 1.41 | 3.43E-02 | 0.93 | 6.23E-02 |
| AT5G08080 | GQS(ph)SREGDVELGEQQGGDQGLEDFFK | -1.40 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.69 | 4.63E-02 |
| AT5G08080 | GQS(ph)SREGDVELGEQQGGDQGLEDFFKK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 4.63E-02 |
| AT5G12370 | SSS(ph)VNSVPLILDIEDFK | -1.26 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.38 | 4.06E-01 |
| AT5G16880 | ISAGVSSM(ox)S(ph)FK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G46860 | (ac)S(ph)FQDLESGR | 0.23 | 5.97E-01 | 1.51 | 3.20E-03 | -1.12 | 1.82E-02 | 0.17 | 5.50E-01 |
| AT5G58440 | S(ph)PSSSSSDYIK | 0.07 | 9.24E-01 | 0.56 | 5.28E-01 | -0.49 | 5.98E-01 | 0.00 | 9.87E-01 |
| AT5G63640 | ESQSVS(ph)PSSILQK | | 1.00E+00 | | 4.05E-02 | | 1.00E+00 | | 4.83E-01 |

## development.

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G13980 | LELFPDQESSQLGDDETVSNGLS(ph)SPENTTGS | | 1.00E+00 | | 1.00E+00 | | 3.61E-02 | | 1.00E+00 |
| AT1G13980 | LELFPDQESSQLGDDETVSNGLSS(ph)PENTTGS | | 1.00E+00 | | 1.00E+00 | | 3.61E-02 | | 1.00E+00 |
| AT1G15750 | APS(ph)PVNNPLLGGIPK | 0.14 | 4.08E-01 | 0.29 | 3.74E-01 | 0.23 | 4.84E-01 | 0.38 | 4.46E-01 |
| AT1G32400 | EKYGLDTSEFTYNPS(ph)ESHR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G32400 | QAAPVTGVPVAPTLDQRPS(ph)R | -0.99 | 9.71E-02 | 1.15 | 2.74E-02 | -0.54 | 2.01E-01 | 1.60 | 1.65E-02 |
| AT1G32400 | QAAPVTGVPVAPTLDQRPS(ph)RSDPWSAR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G32400 | YGLDTSEFTYNPSES(ph)HR | -0.43 | 5.69E-01 | -0.10 | 9.69E-01 | 1.20 | 4.14E-01 | 1.52 | 2.19E-01 |
| AT1G48410 | FYM(ox)EPET(ph)SDSGSM(ox)ASGSM(ox)AR | -0.68 | 1.00E+00 | -0.34 | 3.88E-01 | -0.39 | 1.33E-01 | -0.05 | 1.00E+00 |
| AT1G72410 | NLS(ph)DLSLTDDSK | -0.03 | 9.78E-01 | 0.89 | 1.93E-02 | -0.63 | 4.74E-02 | 0.29 | 3.37E-01 |
| AT1G75500 | APVSRNS(ph)IK | | 1.00E+00 | 0.88 | 3.63E-01 | -1.03 | 1.63E-01 | | 1.00E+00 |
| AT1G79280 | RAPS(ph)PGGGSSTIVTLADR | | 1.00E+00 | | 2.50E-01 | | 1.00E+00 | | 1.72E-01 |
| AT1G79350 | FVEENYPLPEQPEPLS(ph)EDDSVK | 0.15 | 2.19E-01 | -0.20 | 3.65E-02 | 0.81 | 5.03E-03 | 0.46 | 6.93E-03 |
| AT1G79350 | FVEENYPLPEQPEPLS(ph)EDDSVKELQR | | 1.00E+00 | | 3.65E-02 | | 5.03E-03 | | 1.00E+00 |
| AT1G80490 | APS(ph)PVNNPLLGSLPK | 0.00 | 5.28E-01 | 0.25 | 4.80E-01 | 0.18 | 6.24E-01 | 0.43 | 5.82E-01 |
| AT1G80530 | SS(ph)PLGSSDNLAK | 0.35 | 4.20E-01 | 0.88 | 9.42E-02 | -0.79 | 1.27E-01 | -0.27 | 5.35E-01 |
| AT1G80530 | SSPLGS(ph)SDNLAK | 0.01 | 9.27E-01 | 0.14 | 7.17E-01 | -0.24 | 4.07E-01 | -0.11 | 7.37E-01 |
| AT2G19520 | (ac)M(ox)ESDEAAAVS(ph)PQATTPSGGTGASGPK | 0.19 | 5.70E-01 | 0.83 | 2.03E-02 | -1.02 | 7.48E-03 | -0.37 | 2.85E-01 |
| AT2G20330 | GLGNINDS(ph)DDEGDM(ox)IGPPPPPPAAR | -0.31 | 1.25E-01 | -0.35 | 5.83E-02 | 0.69 | 7.91E-02 | 0.65 | 2.31E-02 |
| AT2G34350 | TTLLDS(ph)PK | 0.08 | 5.76E-01 | 1.07 | 2.71E-01 | -0.59 | 5.26E-01 | 0.40 | 9.80E-01 |
| AT2G34350 | TTLLDS(ph)PKLNSSSDVK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G15880 | ERPRS(ph)PPTNSLSM(ox)DYQTADSESVLK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G18390 | NLGLGS(ph)DDEDDVEDDEGGGINGGDVKPVTGEER | | 1.00E+00 | | 1.00E+00 | | 1.66E-01 | | 7.44E-03 |
| AT3G28050 | EVALVEDDNKANHEEANEADLDS(ph)PSGSQK | | 1.00E+00 | | 5.78E-02 | | 1.00E+00 | | 1.00E+00 |
| AT3G48740 | AEIEDGQT(ph)PK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G48740 | LGTVSS(ph)PEPISVVR | 0.92 | 9.34E-03 | 0.64 | 4.37E-02 | -0.36 | 2.07E-01 | -0.64 | 4.43E-02 |
| AT4G01450 | TNVNNGQLLVIPM(ox)T(ph)P | 1.65 | 7.17E-02 | 1.60 | 8.08E-03 | -0.31 | 3.53E-01 | -0.36 | 2.43E-01 |
| AT4G11270 | AAS(ph)LSTSKPSSSQEK | -0.14 | 9.19E-01 | -0.08 | 8.77E-01 | -0.51 | 3.82E-01 | -0.45 | 5.25E-01 |
| AT4G11270 | FDFLHGIDS(ph)PASS(ph)PR | -0.73 | 2.11E-01 | -0.57 | 1.71E-01 | -0.75 | 1.00E+00 | -0.59 | 3.68E-01 |
| AT4G11270 | SLS(ph)GISLNEPK | | 1.00E+00 | 0.94 | 4.59E-03 | -0.32 | 1.66E-01 | | 1.00E+00 |
| AT4G29860 | IIQLPQSSPDES(ph)PNASTK | | 1.00E+00 | 0.54 | 2.30E-01 | -0.08 | 3.64E-01 | | 1.00E+00 |
| AT4G33400 | FM(ox)NDNFAIS(ph)GSPEAPLVIATPM(ox)K | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G33400 | S(ph)PSSSLDDVEAK | 0.47 | 5.36E-01 | 0.60 | 2.57E-01 | -0.04 | 9.36E-01 | 0.10 | 6.26E-01 |
| AT4G37060 | (ac)MENES(ph)PSKK | 0.55 | 4.76E-01 | | 1.00E+00 | | 1.00E+00 | -0.38 | 2.95E-01 |
| AT4G37060 | DSS(ph)IGSQEIK | 0.25 | 1.06E-01 | 0.22 | 3.15E-01 | -0.33 | 6.05E-02 | -0.36 | 3.10E-01 |
| AT5G14120 | REDQEPGLQT(ph)PDLILS(ph)EVEDEKPK | -0.79 | 3.24E-01 | | 1.00E+00 | | 1.00E+00 | 0.94 | 5.64E-01 |
| AT5G16780 | ADYEGS(ph)PVR | | 1.00E+00 | 0.10 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G16780 | ADYEGS(ph)PVREHR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.47 | 1.00E+00 |
| AT5G46750 | QEAAVVSS(ph)PK | 3.30 | 3.13E-02 | 0.20 | 2.42E-01 | -0.58 | 1.53E-01 | -3.68 | 3.49E-03 |
| AT5G63420 | AEGKENSRDDDELADAS(ph)DSETK | | 2.57E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |

## protein.degradation

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G09730 | LSS(ph)PTGEAEEMEK | 0.49 | 3.40E-01 | 0.42 | 9.07E-02 | -0.32 | 1.71E-01 | -0.39 | 3.96E-01 |
| AT1G12760 | RNS(ph)GVQDLSLGHLDTESSSVAK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G20200 | DNNTPS(ph)QSIISSSTSTM(ox)QNLK | 0.45 | 6.08E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G20200 | TQDVEM(ox)KDNNTPS(ph)QSIISSSTSTM(ox)QNLK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G27750 | GLLNQHT(ph)PS(ph)PSAR | | 1.00E+00 | 0.68 | 7.03E-02 | -0.45 | 1.08E-01 | | 1.00E+00 |
| AT1G43690 | SKPIEEEETGSGSQSGGES(ph)PEAK | -0.05 | 8.76E-01 | 0.32 | 7.75E-01 | -0.44 | 5.85E-01 | -0.06 | 9.12E-01 |
| AT1G43910 | GEDS(ph)SVEEEGEIEDAETK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G43910 | KGEDS(ph)SVEEEGEIEDAETK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G54710 | NAELTVDT(ph)SDSEDQTKPLEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G68070 | (ac)SSPES(ph)PSGSDSSTPLLR | 0.16 | 6.68E-01 | | 1.00E+00 | | 1.00E+00 | -0.14 | 6.81E-01 |
| AT1G68070 | SRPGDLEAAQATNQDS(ph)EDEDNDER | -0.42 | 3.18E-01 | 0.75 | 1.21E-01 | -1.20 | 2.66E-02 | -0.03 | 9.84E-01 |
| AT1G70320 | ASDNSVSASSS(ph)TAERESDEDSSNALAVR | | 1.00E+00 | | 4.95E-02 | | 2.42E-01 | | 1.00E+00 |
| AT1G70320 | ASDNSVSASSSTAERES(ph)DEDSSNALAVR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G75990 | DNQTPTQSVVS(ph)APTSTLQNLK | -0.09 | 6.90E-01 | | 1.00E+00 | 0.62 | 6.73E-01 | | 1.00E+00 |
| AT2G01470 | IIMDPEKS(ph)EDDDESSSSSSSSR | 0.57 | 1.73E-01 | -0.42 | 2.89E-01 | 0.61 | 1.37E-01 | -0.38 | 3.54E-01 |
| AT2G21470 | ETENVES(ph)EDDDIM(ox)EVENPM(ox)M(ox)VSK | | 1.00E+00 | | 1.00E+00 | -0.09 | 1.00E+00 | | 1.00E+00 |
| AT2G23140 | DLSDFS(ph)PK | -0.08 | 3.21E-01 | 0.99 | 1.05E-03 | -0.99 | 1.72E-03 | 0.08 | 4.89E-02 |
| AT2G23140 | EGASPS(ph)RPASALGASS(ph)PGISGNGYGLDAR | 0.40 | 2.66E-01 | -0.29 | 1.18E-01 | -0.30 | 2.66E-01 | -0.98 | 3.55E-02 |
| AT2G27210 | LIHPLPPAITS(ph)PET(ph)SPER | 1.47 | 5.84E-02 | 1.65 | 1.67E-02 | 0.41 | 1.47E-01 | 0.58 | 1.90E-01 |
| AT2G27210 | LILFGGATALEGNSGGTGT(ph)PTSAGSAGIR | -0.08 | 3.85E-01 | | 1.00E+00 | | 1.00E+00 | 0.32 | 4.33E-01 |
| AT2G32730 | ASTEKEGDSM(ox)QVDS(ph)PAAVEK | -0.58 | 3.41E-01 | -0.20 | 3.36E-01 | -0.52 | 2.37E-01 | -0.14 | 8.27E-01 |
| AT2G32730 | EGDSMQVDS(ph)PAAVEK | 1.31 | 3.18E-01 | 0.98 | 9.93E-04 | -0.22 | 2.61E-01 | -0.55 | 3.16E-03 |
| AT2G35330 | ALT(ph)LM(ox)GCTVR | | 4.80E-01 | | 1.00E+00 | | 1.00E+00 | | 6.86E-03 |
| AT3G02290 | VM(ox)EFNET(ph)P | 0.25 | 3.50E-02 | 0.28 | 7.77E-01 | -0.23 | 3.81E-01 | -0.20 | 8.74E-01 |
| AT3G05200 | TNS(ph)LLVLPR | 0.43 | 1.27E-01 | 0.98 | 4.11E-02 | -0.59 | 1.14E-01 | -0.05 | 4.49E-01 |
| AT3G51800 | (ac)SS(ph)DDERDEKELSLTSPEVVTK | -0.24 | 3.59E-01 | -0.58 | 7.61E-01 | 0.57 | 7.81E-01 | 0.23 | 3.46E-01 |
| AT3G58460 | LLEDSSSPDRLS(ph)DATVNTVADSR | -1.16 | 5.71E-02 | | 1.00E+00 | | 1.00E+00 | 0.88 | 6.42E-02 |
| AT3G62240 | YLQAVGS(ph)FGGGGSR | | 1.00E+00 | 0.08 | 8.45E-01 | | 1.00E+00 | | 1.00E+00 |
| AT4G00752 | YSLDNNPSSVLS(ph)PR | 0.67 | 3.03E-01 | 0.93 | 9.32E-02 | 0.02 | 9.17E-01 | 0.28 | 4.38E-01 |

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT4G04210 | TLS(ph)DLNR | 0.44 | 3.12E-01 | 0.68 | 2.15E-01 | -1.40 | 3.19E-02 | -1.16 | 6.51E-02 |
| AT4G30890 | LDASRPAS(ph)SDKNNDSDAK | 0.91 | 5.10E-02 | | 1.00E+00 | | 1.00E+00 | -0.96 | 4.73E-02 |
| AT4G30890 | TDPIGLDNLSM(ox)S(ph)DGESDPVYK | 0.01 | 8.11E-01 | -0.28 | 7.09E-02 | 0.04 | 7.16E-01 | -0.25 | 9.11E-02 |
| AT4G38600 | SESALKPAAPIGNTEPGTLPSGAGVS(ph)SPSSSTPASTTR | | 2.09E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G01450 | (ac)SLPDSLPSSS(ph)SSPPVTR | | 1.00E+00 | | 1.67E-02 | | 5.90E-03 | | 1.00E+00 |
| AT5G22030 | SNS(ph)LSFLGK | 0.42 | 1.21E-02 | 0.98 | 4.09E-03 | -0.58 | 4.26E-02 | -0.02 | 4.93E-01 |
| AT5G26860 | AVES(ph)DSEVSDSK | 1.48 | 6.74E-03 | 0.91 | 4.19E-02 | -0.22 | 5.27E-01 | -0.79 | 1.07E-01 |
| AT5G55160 | (ac)S(ph)ATPEEDKK | -0.75 | 6.46E-02 | 0.61 | 4.26E-01 | -0.16 | 9.51E-01 | 1.20 | 6.87E-03 |

## protein.posttranslational modification

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G01540 | ADFASAAIAT(ph)PPIS(ph)KEIK | | 1.00E+00 | 0.10 | 3.06E-01 | -0.56 | 2.65E-01 | | 1.00E+00 |
| AT1G03080 | LNS(ph)DLQK | | 1.00E+00 | | 2.05E-02 | | 1.39E-03 | | 1.00E+00 |
| AT1G03740 | AS(ph)SAVVDSDLDIDPK | 0.65 | 1.77E-01 | 0.50 | 2.75E-01 | -0.36 | 4.96E-01 | -0.52 | 2.33E-01 |
| AT1G08420 | LIHPLPPALS(ph)SPETSPER | 0.45 | 6.17E-01 | 0.27 | 8.93E-01 | 0.90 | 1.48E-01 | 0.73 | 3.93E-01 |
| AT1G08420 | LVLFGGATALEGNSGGTGT(ph)PTSAGSAGIR | -1.22 | 2.55E-01 | | 1.00E+00 | | 1.00E+00 | -0.07 | 7.98E-01 |
| AT1G08420 | QLS(ph)IDQFENEGR | 0.27 | 3.22E-01 | 0.31 | 5.85E-01 | -0.29 | 6.25E-01 | -0.25 | 3.49E-01 |
| AT1G08420 | QLS(ph)IDQFENEGRR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G08420 | QM(ox)S(ph)INSVPK | | 1.00E+00 | 0.80 | 2.85E-03 | -0.77 | 5.78E-02 | | 1.00E+00 |
| AT1G11330 | M(ox)EALTS(ph)DNESASNQIK | | 1.00E+00 | 1.06 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G11330 | MEALTS(ph)DNESASNQIK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G14000 | (ac)SSDSPAAGDGGEQAAAGTSVPS(ph)PSYDK | 0.67 | 1.43E-01 | 1.05 | 7.16E-02 | -0.25 | 6.59E-01 | 0.12 | 9.11E-01 |
| AT1G14000 | (ac)SSDSPAAGDGGEQAAAGTSVPS(ph)PSYDKQK | -0.11 | 7.58E-01 | 0.54 | 3.73E-03 | -0.53 | 6.37E-02 | 0.12 | 6.98E-01 |
| AT1G16270 | TVS(ph)GGGIETEAR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G22280 | TDQAILSNS(ph)SDLGR | 0.83 | 1.32E-01 | 0.76 | 4.50E-01 | -0.43 | 6.94E-01 | -0.49 | 2.33E-01 |
| AT1G34750 | AFGDKS(ph)LK | | 1.00E+00 | 0.25 | 2.47E-01 | -0.18 | 9.62E-02 | | 1.00E+00 |
| AT1G34750 | TDQAILSHS(ph)SDLGR | | 1.00E+00 | | 2.47E-01 | | 9.62E-02 | | 1.00E+00 |
| AT1G48490 | DSLAAVES(ph)PEGM(ox)K | | 1.00E+00 | | 3.88E-01 | | 1.33E-01 | | 1.00E+00 |
| AT1G53165 | EFS(ph)SNANFSPLAR | | 1.00E+00 | -0.69 | 4.75E-02 | 1.03 | 2.73E-02 | | 1.00E+00 |
| AT1G67580 | NTSQT(ph)PEVGELVR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G67580 | S(ph)PDPLEEQR | 1.19 | 5.71E-02 | -0.01 | 4.75E-01 | 0.09 | 4.80E-01 | -1.11 | 1.01E-01 |
| AT1G67580 | WAAGNS(ph)SPTDEVEIVEEVGEK | -0.99 | 1.14E-01 | | 1.00E+00 | | 1.00E+00 | -0.21 | 1.00E+00 |
| AT1G69220 | M(ox)STTS(ph)LPDSITR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G71530 | GDGDLQLT(ph)SR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G71860 | FDLSSADS(ph)PPSK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G73450 | HPWLSYPYEPIS(ph)A | | 6.08E-02 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G74330 | S(ph)NSFAWAK | | 1.00E+00 | 0.19 | 5.13E-01 | -0.19 | 4.48E-01 | | 1.00E+00 |
| AT1G79570 | LS(ph)KSDNSLSSQFVTSESPANTAQQDSGK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G79570 | NSTLLGLDS(ph)SSANNLAELDVR | -0.30 | 6.64E-01 | | 1.00E+00 | | 1.00E+00 | 0.12 | 7.55E-01 |
| AT1G79570 | NTLVS(ph)GGVR | 0.25 | 7.52E-02 | -0.06 | 8.47E-01 | -0.08 | 7.18E-01 | -0.39 | 1.71E-01 |
| AT1G79570 | TPDS(ph)EPKDEKTETR | 0.63 | 2.41E-01 | -0.21 | 8.95E-01 | 0.43 | 3.91E-01 | -0.40 | 5.77E-01 |
| AT2G05940 | NGVNS(ph)PLR | | 1.00E+00 | | 2.66E-01 | | 4.10E-02 | | 1.00E+00 |
| AT2G17700 | AVVAS(ph)PSQENPR | 1.46 | 2.33E-02 | 0.19 | 7.59E-01 | 0.24 | 5.49E-01 | -1.03 | 6.07E-02 |
| AT2G17700 | VQIESGVM(ox)T(ph)AETGTYR | -0.32 | 2.73E-02 | 0.91 | 4.71E-02 | -0.54 | 4.09E-01 | 0.70 | 2.70E-02 |
| AT2G20050 | VS(ph)SQFLPPDGSR | | 1.00E+00 | 0.23 | 1.83E-01 | -0.60 | 1.49E-02 | | 1.00E+00 |
| AT2G22560 | LIDEFALSGS(ph)K | 1.36 | 1.50E-01 | 0.13 | 8.85E-01 | 0.12 | 6.14E-01 | -1.12 | 2.35E-01 |
| AT2G35050 | GAES(ph)TDATLNAGVPLIDFM(ox)AADSGM(ox)R | -0.67 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.24 | 1.97E-01 |
| AT2G37550 | SKS(ph)SEDIYSR | | 8.33E-02 | | 1.00E+00 | | 1.00E+00 | | 5.55E-02 |
| AT2G40270 | TGLS(ph)GQLQK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G40730 | GKPLEQAPLASSSS(ph)APSLAAAASNATSTATEAPSVK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G40730 | TVQS(ph)DDEDPWAAIAAPPPTTR | 0.66 | 1.35E-01 | -0.54 | 9.52E-02 | 1.22 | 3.86E-02 | 0.02 | 8.71E-01 |
| AT3G01490 | SLS(ph)DGEDNVNNTR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G04910 | TTS(ph)LPVDAIDS | -0.39 | 1.03E-01 | 0.76 | 4.59E-02 | -0.85 | 2.87E-02 | 0.30 | 7.22E-02 |
| AT3G09830 | IVEASSGNGS(ph)PQLVPLNSVK | 0.64 | 1.04E-01 | -0.30 | 2.17E-01 | 0.63 | 4.95E-02 | -0.31 | 3.88E-01 |
| AT3G10540 | LAPDPASQS(ph)ASPER | -0.54 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.05 | 4.16E-01 |
| AT3G10540 | LAS(ph)IDSFDSR | 0.87 | 2.29E-02 | 0.94 | 1.18E-02 | -0.34 | 3.07E-01 | -0.27 | 4.14E-01 |
| AT3G17420 | FVEDIENGDKFS(ph)GSLEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G17420 | SNATT(ph)LPVTQSPR | 0.95 | 0.00E+00 | | 1.00E+00 | -0.05 | 0.00E+00 | | 1.00E+00 |
| AT3G17850 | SLS(ph)PTLPPSGSR | -0.11 | 7.25E-01 | 0.73 | 9.92E-02 | -0.57 | 1.47E-01 | 0.27 | 4.72E-01 |
| AT3G19420 | (ac)SSESPNLPAAAGTVPDNHPPPPPVVTAAEAGSDDS(ph)PKGVASK | -0.52 | 8.27E-01 | 0.97 | 1.00E+00 | -0.58 | 1.06E-01 | 0.91 | 3.18E-01 |
| AT3G19420 | ETENPDKDDVFS(ph)DNEGDSTGPTK | 0.69 | 1.93E-01 | 0.75 | 2.60E-01 | -0.15 | 6.73E-01 | -0.09 | 5.91E-01 |
| AT3G19420 | VM(ox)AADASVFSFGDEDDFES(ph)D | -0.51 | 4.73E-01 | -0.76 | 5.41E-03 | 1.12 | 1.56E-02 | 0.88 | 1.13E-01 |
| AT3G22750 | NETKAS(ph)PENNLR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G25840 | NEVTPYLVS(ph)R | 0.55 | 1.00E+00 | 0.57 | 7.55E-02 | -0.39 | 1.10E-01 | -0.36 | 7.03E-02 |
| AT3G25840 | NQAQAGLGEGS(ph)PK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G25840 | TADS(ph)DGES(ph)GEIKFEDNNLPPLGEK | -1.49 | 4.16E-02 | | 1.00E+00 | | 1.00E+00 | 1.12 | 1.41E-01 |
| AT3G26700 | NVAIELS(ph)M(ox)R | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G48190 | SLAPDS(ph)PEVGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G54030 | SASVLES(ph)PDIENGGK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G55270 | FSSLSLLPS(ph)QTSPK | -2.75 | 1.82E-03 | -0.10 | 1.84E-01 | -2.92 | 1.13E-03 | -0.27 | 2.22E-01 |
| AT3G55270 | FSSLSLLPSQTS(ph)PKESR | | 1.00E+00 | -2.31 | 3.19E-02 | 1.38 | 1.00E+00 | | 1.00E+00 |
| AT3G55270 | GVNTFLQPS(ph)PNR | | 1.00E+00 | -0.17 | 6.87E-01 | | 1.00E+00 | -0.02 | 9.26E-01 |
| AT3G58640 | AISLPSS(ph)PQNYR | | 1.00E+00 | -0.66 | 2.54E-02 | 0.65 | 1.00E+00 | | 1.00E+00 |
| AT3G58640 | SIS(ph)ITPEIGDDIVR | 0.08 | 6.84E-01 | -0.72 | 8.14E-02 | 0.77 | 6.32E-02 | -0.03 | 8.01E-01 |
| AT3G58760 | DYVNPGGS(ph)NR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G63260 | (ac)AS(ph)GGGEADKSLEIGSGTADPK | -0.08 | 7.82E-01 | 0.37 | 3.31E-01 | -0.28 | 4.89E-01 | 0.17 | 5.87E-01 |
| AT4G03080 | QLS(ph)LDQFQNESR | 2.31 | 2.34E-03 | 0.87 | 3.58E-02 | -0.87 | 4.04E-02 | -2.31 | 6.92E-03 |
| AT4G10730 | ASANSLS(ph)APIK | 0.05 | 6.97E-01 | 0.21 | 5.34E-01 | -0.18 | 6.25E-01 | -0.03 | 8.00E-01 |
| AT4G10730 | RAPS(ph)FSGPLNLPNR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G10730 | SAS(ph)VGNWILDSK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G14350 | M(ox)LAYS(ph)TVGTPDYIAPEVLLK | -0.61 | 3.18E-01 | -1.03 | 1.28E-01 | 1.06 | 2.47E-01 | 0.64 | 4.22E-01 |
| AT4G22130 | SLPLSGT(ph)PEVQEQR | 1.38 | 1.96E-02 | -0.62 | 8.01E-02 | 1.03 | 3.46E-02 | -0.97 | 3.54E-02 |
| AT4G24100 | ASSNSLS(ph)APIK | -0.52 | 4.09E-01 | -0.32 | 2.15E-01 | 0.24 | 3.53E-01 | 0.44 | 4.46E-01 |
| AT4G24100 | RAPS(ph)FSGPLNLSTR | | 1.00E+00 | | 2.15E-01 | | 3.53E-01 | | 1.00E+00 |
| AT4G24100 | S(ph)DSNGNVEPVASER | 0.10 | 8.51E-02 | 0.38 | 5.54E-02 | -0.59 | 1.00E+00 | -0.31 | 2.11E-01 |
| AT4G29810 | FLTQSGT(ph)FK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G29810 | IISQLEPEVLS(ph)PIKPADDQLSLSDLDM(ox)VK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G35230 | SYS(ph)TNLAYTPPEYLR | 0.04 | 7.15E-01 | -0.58 | 2.72E-02 | 0.71 | 9.67E-03 | 0.09 | 8.83E-01 |
| AT4G35600 | VGS(ph)GMIVAIK | | 1.00E+00 | 1.22 | 4.29E-02 | -0.31 | 5.56E-01 | | 1.00E+00 |
| AT4G38470 | AQTGVM(ox)T(ph)AETGTYR | 0.05 | 3.68E-01 | | 3.91E-01 | | 3.23E-01 | -0.34 | 1.03E-01 |
| AT5G13160 | LNPVDES(ph)NHGQK | 2.23 | 3.75E-03 | -0.54 | 2.84E-01 | 0.66 | 4.10E-02 | -2.11 | 8.38E-02 |
| AT5G13530 | VGFPGAS(ph)R | 0.21 | 4.70E-01 | 0.10 | 7.04E-01 | -0.07 | 8.35E-01 | -0.19 | 5.19E-01 |
| AT5G14720 | FKVTSADLS(ph)PK | | 1.00E+00 | -0.26 | 2.01E-01 | | 1.01E-02 | | 1.00E+00 |
| AT5G14720 | QDESALS(ph)PER | 0.72 | 1.58E-01 | 0.11 | 8.81E-01 | 0.18 | 5.47E-01 | -0.43 | 4.10E-01 |
| AT5G14720 | QPGS(ph)PETNVDDLLQTPPATSR | 0.96 | 1.42E-01 | -0.78 | 1.78E-01 | 0.97 | 1.25E-01 | -0.76 | 1.94E-01 |
| AT5G14720 | SYS(ph)GSLYR | -0.76 | 3.07E-02 | 0.69 | 5.27E-02 | -0.72 | 3.39E-02 | 0.73 | 1.00E+00 |
| AT5G14720 | TQAALIS(ph)DDDTSHAEEPDFNQK | 0.83 | 6.52E-02 | 0.13 | 8.32E-01 | 1.01 | 8.26E-02 | 0.31 | 6.75E-01 |

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT5G14720 | VTSADLS(ph)PK | 0.80 | 7.92E-03 | 0.11 | 7.22E-01 | -0.18 | 5.59E-01 | -0.87 | 1.62E-02 |
| AT5G18500 | S(ph)SSNLIPVSR | -0.28 | 2.73E-01 | 0.34 | 9.18E-02 | -0.34 | 1.73E-01 | 0.27 | 1.35E-01 |
| AT5G22840 | EEEEEAS(ph)DEDKDKEK | -2.18 | 4.93E-02 | | 1.00E+00 | -1.98 | 1.27E-01 | | 1.00E+00 |
| AT5G23720 | NAGLS(ph)SSSLK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G41260 | S(ph)NPDVTGLDEEGR | 0.20 | 5.99E-01 | 1.08 | 3.21E-02 | -0.78 | 5.98E-02 | 0.10 | 8.35E-01 |
| AT5G41260 | S(ph)NPDVTGLDEEGRGESNDLPQFR | -0.93 | 3.64E-01 | -1.07 | 1.02E-01 | 1.12 | 9.58E-02 | 0.98 | 3.43E-01 |
| AT5G41260 | SYS(ph)TNLAFTPPEYLR | 1.07 | 1.58E-01 | -1.93 | 3.48E-02 | 2.62 | 1.47E-02 | -0.39 | 4.09E-01 |
| AT5G46570 | TANLPSSDDPSAPNKPES(ph)VNGDQVDQEIQNFK | -0.43 | 3.96E-01 | | 1.00E+00 | | 1.00E+00 | 0.33 | 6.04E-01 |
| AT5G47070 | SETSS(ph)FNLQTPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G50000 | ALT(ph)LEK | | 1.00E+00 | 0.01 | 1.00E+00 | -0.20 | 7.25E-02 | | 1.00E+00 |
| AT5G55910 | SM(ox)S(ph)FVGTHEYLAPEIIK | -2.02 | 1.00E+00 | | 1.00E+00 | | 2.51E-02 | 1.64 | 1.00E+00 |
| AT5G57020 | (ac)ADNNS(ph)PPGSVEQK | 1.14 | 1.02E-01 | 0.33 | 6.39E-02 | 0.43 | 2.41E-01 | -0.38 | 5.69E-02 |
| AT5G57610 | LQQYPDS(ph)PR | 1.13 | 3.07E-03 | 0.12 | 2.83E-01 | 0.49 | 1.62E-02 | -0.53 | 8.69E-02 |
| AT5G57610 | TFQEFPS(ph)SPSSAR | 2.62 | 1.98E-02 | 0.97 | 3.27E-02 | -0.39 | 3.78E-01 | -2.05 | 2.21E-03 |
| AT5G57610 | VGS(ph)GQM(ox)LAQR | 0.22 | 6.61E-01 | -0.32 | 5.27E-01 | -0.09 | 8.19E-01 | -0.63 | 2.19E-01 |
| AT5G58140 | APPS(ph)PLNDAESLSER | | 1.00E+00 | 0.81 | 7.22E-02 | -0.46 | 3.04E-01 | | 1.00E+00 |
| AT5G58140 | S(ph)LEIFNPSSGK | | 1.00E+00 | 0.64 | 2.40E-01 | -0.21 | 7.20E-01 | | 1.00E+00 |
| AT5G58950 | SVS(ph)PSPQM(ox)AVPDVFK | -0.67 | 9.47E-03 | -0.41 | 1.75E-01 | -0.01 | 6.99E-01 | 0.24 | 6.23E-02 |
| AT5G66880 | S(ph)TVGTPAYIAPEVLLR | -0.09 | 7.45E-01 | | 1.00E+00 | | 1.00E+00 | 1.00 | 7.98E-01 |

## RNA.regulation of transcription

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G02080 | QIDLPLDVANS(ph)PNTDVPSK | 0.26 | 6.63E-01 | 0.41 | 2.71E-01 | 0.02 | 4.83E-01 | 0.17 | 6.12E-01 |
| AT1G04950 | LSVDSSENQS(ph)PQK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G04950 | LSVDSSENQS(ph)PQKR | 0.12 | 3.40E-01 | -0.04 | 4.97E-01 | -0.43 | 6.91E-02 | -0.59 | 8.36E-02 |
| AT1G05805 | SQLS(ph)FTNHDSLAR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G07000 | APDS(ph)FDSDDEFPGEEDNDTSDGVIVAR | 1.08 | 1.47E-01 | -0.46 | 2.42E-01 | 0.94 | 1.67E-01 | -0.59 | 1.97E-01 |
| AT1G07000 | APDSFDS(ph)DDEFPGEEDNDTSDGVIVARPITDYK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 9.04E-02 |
| AT1G08680 | ASDYSVSS(ph)AGDPFR | | 1.00E+00 | 0.86 | 6.33E-02 | -0.87 | 9.45E-02 | | 1.00E+00 |
| AT1G08680 | ASS(ph)FVYS(ph)PGR | 0.41 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.09 | 1.48E-01 |
| AT1G08680 | SDIQS(ph)PNFQQEAEFR | 0.27 | 3.02E-01 | 0.07 | 8.74E-01 | 0.08 | 6.39E-01 | -0.12 | 5.92E-01 |
| AT1G14510 | MS(ph)PPPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G20110 | SIS(ph)FSSSGR | -0.10 | 8.27E-01 | 0.66 | 1.28E-01 | -0.81 | 7.24E-02 | -0.05 | 8.67E-01 |
| AT1G20670 | ATDILQGS(ph)PVESGPTTPLPDKK | -0.44 | 6.70E-01 | -0.40 | 4.62E-01 | 0.24 | 6.58E-01 | 0.27 | 9.28E-01 |
| AT1G20670 | KDFENLRQDS(ph)DDEEPQSQQQQQQPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G20696 | LEEGPKEDEESDKSVSEVNDEDDAEDGS(ph)EEEEDDD | 0.01 | 4.62E-01 | 0.44 | 7.50E-02 | 0.01 | 9.70E-01 | 0.45 | 1.27E-02 |
| AT1G20696 | SLS(ph)DSEKAPYVAK | -0.89 | 1.38E-01 | -0.36 | 5.18E-01 | -1.01 | 1.04E-01 | -0.48 | 2.44E-01 |
| AT1G20696 | SVSEVNDEDDAEDGS(ph)EEEEDDD | | 1.00E+00 | 0.29 | 5.95E-02 | 0.19 | 1.00E+00 | | 1.00E+00 |
| AT1G29220 | LLDGVGASSS(ph)AHGT(ph)PR | 0.34 | 2.67E-01 | | 1.00E+00 | | 1.00E+00 | -0.45 | 1.19E-01 |
| AT1G32130 | YGGDAGDRS(ph)PTHYPQAEEGEDEDEVNNLFK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G32130 | YGGDAGDRSPT(ph)HYPQAEEGEDEDEVNNLFK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G33240 | SAAFEIAFQS(ph)PANR | | 7.58E-02 | | 1.00E+00 | | 1.00E+00 | | 4.84E-02 |
| AT1G51140 | AM(ox)S(ph)PISEVDVKPGFSSR | -0.74 | 2.81E-01 | 0.20 | 6.98E-01 | 0.26 | 6.94E-01 | 1.20 | 1.17E-01 |
| AT1G51140 | TLS(ph)GGFNR | 1.39 | 8.81E-02 | 0.95 | 1.23E-01 | -0.20 | 3.78E-01 | -0.63 | 1.71E-01 |
| AT1G51140 | TQS(ph)GGLDQYK | 0.67 | 4.75E-02 | | 1.00E+00 | | 1.00E+00 | -0.70 | 1.85E-01 |
| AT1G55110 | NQPGNPDPEAEVMALS(ph)PK | 0.23 | 6.46E-01 | -0.49 | 1.00E+00 | 0.46 | 1.54E-01 | -0.26 | 3.09E-01 |
| AT1G56460 | AFDDADYVKDDDEEEEEAVS(ph)DVELENK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.99 | 1.00E+00 |
| AT1G61730 | IKS(ph)PSATTAAAPPAK | 0.98 | 9.99E-02 | -0.04 | 9.96E-01 | 0.02 | 9.96E-01 | -1.00 | 7.01E-02 |
| AT1G62300 | LGREES(ph)PETESNK | 0.66 | 2.30E-01 | -0.39 | 3.41E-01 | 1.11 | 1.21E-02 | 0.06 | 9.23E-01 |
| AT1G63850 | S(ph)PGNQFNDPNSSDVK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G65440 | EQGNGQGES(ph)S(ph)DDEFDSR | | 9.67E-02 | | 1.00E+00 | | 1.00E+00 | | 1.19E-01 |
| AT1G67325 | SNGS(ph)PSRAPEENDQ | 0.55 | 4.15E-01 | 0.19 | 9.81E-01 | 0.10 | 5.85E-01 | -0.26 | 7.50E-01 |
| AT1G67590 | ATTPVGRS(ph)PVTS(ph)PVR | | 1.14E-01 | 0.57 | 1.00E+00 | -0.09 | 1.00E+00 | | 1.00E+00 |
| AT1G73150 | AES(ph)M(ox)TNPVKPAVLPVVPEK | -2.67 | 4.19E-02 | | 1.00E+00 | | 1.00E+00 | 0.42 | 2.38E-01 |
| AT1G73150 | AES(ph)MTNPVKPAVLPVVPEK | -1.01 | 6.08E-02 | | 1.00E+00 | | 1.00E+00 | 1.76 | 1.00E+00 |
| AT1G76880 | S(ph)PPPQPPAPLPQPIQAVVSTLDTTK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.37 | 1.00E+00 |
| AT2G02070 | TPNSDAEVIALS(ph)PK | 0.45 | 1.27E-01 | 0.69 | 4.00E-03 | -0.70 | 4.94E-03 | -0.46 | 1.15E-01 |
| AT2G02080 | NQPGNPNPDAEVVALS(ph)PK | -0.27 | 5.34E-01 | 0.15 | 9.95E-01 | -0.63 | 2.47E-01 | -0.21 | 7.41E-01 |
| AT2G04880 | LVPHTVASQSEVDVAS(ph)PVSEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G17410 | RSFLLDDASDGNES(ph)GTEEDQSAFM(ox)K | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.44 | 2.30E-01 |
| AT2G17410 | SFLLDDASDGNES(ph)GTEEDQSAFM(ox)K | -0.29 | 2.91E-01 | 0.12 | 8.53E-01 | 0.14 | 5.19E-01 | 0.55 | 7.20E-02 |
| AT2G17410 | SFLLDDASDGNES(ph)GTEEDQSAFMK | 0.08 | 7.46E-01 | -0.88 | 8.15E-02 | 1.60 | 5.93E-03 | 0.65 | 3.15E-01 |
| AT2G17560 | EEDDSDKSKSEVDEAVS(ph)EEEAEDDD | | 1.00E+00 | 0.49 | 1.05E-01 | -0.42 | 9.72E-02 | | 1.00E+00 |
| AT2G17560 | LASGTNREEDDS(ph)DKSK | -2.58 | 1.18E-01 | | 1.00E+00 | -1.63 | 3.36E-01 | | 1.00E+00 |
| AT2G17560 | SEVDEAVS(ph)EEEAEDDD | 0.42 | 5.18E-01 | 0.78 | 9.01E-02 | -0.13 | 7.85E-01 | 0.23 | 4.37E-01 |
| AT2G22300 | SENTS(ph)PVSGNDSDLSQLSEK | | 1.00E+00 | 1.31 | 6.06E-02 | -0.16 | 3.35E-01 | | 1.00E+00 |
| AT2G23740 | SLGTEGNTEAGVS(ph)PPLDDSR | 1.64 | 1.00E+00 | 2.08 | 1.00E+00 | -0.40 | 3.16E-01 | 0.03 | 1.00E+00 |
| AT2G27040 | FEDQS(ph)ETSSSHGGITAPGPISVAQLPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G27100 | DRS(ph)PLPPPRR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.75 | 1.00E+00 |
| AT2G27100 | S(ph)T(ph)SSSPPPPPPSSSLPQQEQEQDQQQLPLRR | -0.03 | 9.45E-01 | 0.41 | 1.00E+00 | -0.14 | 1.03E-01 | 0.31 | 3.27E-01 |
| AT2G27100 | SGRTS(ph)EPNS(ph)EDEAAGVGKR | 0.18 | 9.67E-01 | -0.50 | 3.06E-01 | -0.33 | 5.41E-01 | -1.01 | 1.08E-01 |
| AT2G27100 | TSEPNS(ph)EDEAAGVGKR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G32080 | TIDS(ph)PGQEETGMTGVSK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G32700 | TEVNLGT(ph)SPR | | 1.00E+00 | -0.12 | 1.00E+00 | -0.34 | 4.87E-02 | | 1.00E+00 |
| AT2G33620 | VAPTQVLM(ox)TPSS(ph)PQSR | 0.67 | 1.69E-01 | 0.31 | 9.07E-02 | -0.46 | 8.63E-02 | -0.81 | 1.10E-01 |
| AT2G38880 | (ac)ADTPS(ph)SPAGDGGESGGSVR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G41070 | QGS(ph)LTLPR | | 1.00E+00 | 1.13 | 2.12E-01 | -0.14 | 1.00E+00 | | 1.00E+00 |
| AT2G41900 | NVVEPIS(ph)PM(ox)SAR | 0.81 | 1.73E-02 | 1.40 | 3.07E-03 | -1.75 | 3.63E-03 | -1.15 | 3.73E-03 |
| AT2G41900 | SM(ox)PPSNLEDLFSAEGS(ph)SSPR | 0.09 | 3.70E-01 | -0.45 | 4.72E-01 | -0.23 | 1.00E+00 | -0.77 | 8.55E-01 |
| AT2G44730 | QGNES(ph)GDDDDHDDGNYTAR | 1.11 | 1.17E-01 | | 1.00E+00 | | 1.00E+00 | -0.88 | 2.17E-01 |
| AT2G45820 | ALAVVEKPIEEHT(ph)PKK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G45820 | ASS(ph)GSADRDVILADLEK | | 1.00E+00 | 0.29 | 3.33E-02 | -0.63 | 8.79E-03 | | 1.00E+00 |
| AT2G45820 | KAS(ph)SGSADRDVILADLEK | | 1.00E+00 | | 3.33E-02 | | 8.79E-03 | | 1.00E+00 |
| AT2G45820 | VDVES(ph)PAVLAPAKEPTPAPVEVADEK | | 1.00E+00 | | 3.33E-02 | | 8.79E-03 | | 1.00E+00 |
| AT2G46020 | LVNEPETEPS(ph)SPQR | 0.57 | 9.89E-02 | 0.57 | 3.42E-01 | -0.37 | 5.84E-01 | -0.36 | 3.31E-01 |
| AT2G46020 | NIDS(ph)GNEEEGDIR | -0.18 | 8.22E-01 | 0.63 | 2.42E-01 | -0.69 | 1.88E-01 | 0.12 | 9.55E-01 |
| AT3G02830 | ASFIAS(ph)PR | | 1.00E+00 | 0.57 | 2.23E-02 | -0.47 | 3.55E-02 | | 1.00E+00 |
| AT3G02830 | VFTYDNTAS(ph)ETDEVVETSTGK | -0.59 | 6.70E-01 | 1.44 | 1.00E+00 | -0.21 | 3.33E-01 | 1.82 | 1.91E-01 |
| AT3G04740 | LIPSLQVVEGVAS(ph)PNKR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G06400 | NSNSDEAFS(ph)S(ph)EEEEER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G09670 | ANLQTEDPGSPVS(ph)PK | -0.39 | 1.20E-01 | -0.09 | 4.66E-01 | 0.22 | 2.04E-01 | 0.52 | 8.96E-02 |
| AT3G13810 | NQPGNPDPESEVIALS(ph)PK | | 1.00E+00 | 0.89 | 9.69E-02 | -0.91 | 1.13E-01 | | 1.00E+00 |
| AT3G18640 | TQVS(ph)PTPIR | 0.98 | 4.00E-02 | | 1.00E+00 | | 1.00E+00 | -0.91 | 5.36E-02 |
| AT3G21810 | DVGLDIVS(ph)DEETNGR | 0.10 | 6.20E-01 | 0.23 | 4.90E-01 | 0.14 | 5.78E-01 | 0.27 | 5.33E-01 |
| AT3G22220 | (ac)M(ox)DS(ph)DLEPVALTPQK | | 1.00E+00 | 0.13 | 2.89E-01 | -0.19 | 1.00E+00 | | 1.00E+00 |
| AT3G24490 | (ac)GDS(ph)EDETGYPKK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G24870 | AQLVSTGGS(ph)PK | 0.84 | 2.13E-02 | -0.24 | 4.59E-01 | -0.17 | 6.87E-01 | -1.25 | 1.35E-02 |

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT3G26910 | EGELS(ph)FDYR | 0.95 | 5.61E-02 | 1.04 | 7.00E-02 | -0.35 | 5.02E-01 | -0.25 | 1.89E-01 |
| AT3G26935 | DGELGELS(ph)PDIR | 0.53 | 8.12E-02 | 0.57 | 5.66E-02 | -0.40 | 1.73E-01 | -0.35 | 2.18E-01 |
| AT3G26935 | SVAGGFM(ox)S(ph)PNM(ox)GK | 0.00 | 9.76E-01 | 0.31 | 3.55E-01 | -0.64 | 1.51E-01 | -0.33 | 4.90E-01 |
| AT3G43590 | HYEESNENDSAT(ph)PER | 1.53 | 8.58E-03 | | 1.00E+00 | | 1.00E+00 | -2.19 | 1.00E+00 |
| AT3G48430 | NPVS(ph)YESEDNGVYQQSGR | | 3.97E-01 | | 1.43E-01 | | 1.00E+00 | | 1.00E+00 |
| AT3G48430 | TYDQEGS(ph)DGHEEAR | 0.21 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.22 | 1.45E-01 |
| AT3G48760 | TVNGGM(ox)SS(ph)PSLQK | 0.18 | 5.85E-01 | 0.47 | 4.24E-01 | -0.89 | 1.48E-01 | -0.60 | 2.17E-01 |
| AT3G51880 | NLEEGS(ph)DES(ph)EKSR | 0.76 | 5.49E-02 | 0.35 | 4.97E-01 | -0.44 | 3.74E-01 | -0.85 | 3.62E-02 |
| AT3G51880 | SEINDEDEAS(ph)GEEELLEK | 0.53 | 3.35E-02 | 0.29 | 4.49E-01 | -0.03 | 7.39E-01 | -0.27 | 2.55E-01 |
| AT3G51880 | SRSEINDEDEAS(ph)GEEELLEK | -0.25 | 7.34E-01 | -0.99 | 2.41E-02 | 0.63 | 1.85E-01 | -0.11 | 6.67E-01 |
| AT3G51950 | ELS(ph)PTGLDS(ph)SPR | | 1.00E+00 | 0.46 | 1.73E-02 | -0.42 | 4.27E-02 | | 1.00E+00 |
| AT3G53340 | (ac)AESQTGGGGGGSHESGGDQS(ph)PR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G54610 | DGALTSNDELESIS(ph)AR | | 1.00E+00 | -0.65 | 1.17E-01 | 0.22 | 4.88E-01 | | 1.00E+00 |
| AT3G61260 | (ac)AEEQKIALESES(ph)PAK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G61260 | IALES(ph)ESPAKVTTPAPADTPAPAPAEIPAPAPAPTPADVTK | -0.56 | 6.53E-01 | -0.45 | 2.35E-01 | 1.00 | 8.32E-02 | 1.11 | 2.17E-01 |
| AT3G61260 | IALESES(ph)PAK | 0.74 | 1.81E-01 | 1.04 | 2.09E-01 | -0.38 | 4.58E-01 | -0.08 | 4.13E-01 |
| AT4G00238 | SGNNEGATES(ph)PAVK | 0.47 | 5.27E-01 | | 1.00E+00 | | 1.00E+00 | -0.86 | 2.42E-01 |
| AT4G06634 | FGENNEDDDDDEET(ph)EYED | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G08350 | APAPVPS(ph)SPGR | 0.50 | 1.14E-01 | 0.50 | 2.62E-01 | 0.28 | 5.65E-01 | 0.28 | 1.00E+00 |
| AT4G08350 | SNFIDDYAEEDS(ph)QEEDDDDEDYGSSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G10710 | EATNADREHGVES(ph)DS(ph)EEER | 0.24 | 4.17E-01 | 0.59 | 1.82E-01 | 0.59 | 1.49E-01 | 0.94 | 7.02E-02 |
| AT4G11560 | FAQVS(ph)S(ph)DEEDDVPITR | -0.01 | 7.49E-01 | 1.03 | 9.60E-02 | -0.14 | 8.85E-01 | 0.90 | 2.10E-01 |
| AT4G14140 | SVDS(ph)DDDVSKER | 0.95 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -1.41 | 1.30E-02 |
| AT4G14540 | (ac)ADSDNDS(ph)GGHKDGGNASTR | -2.64 | 2.96E-03 | | 1.00E+00 | -1.22 | 1.32E-02 | | 1.00E+00 |
| AT4G14920 | TNEVSVLET(ph)TSPSR | | 1.00E+00 | 0.34 | 4.07E-01 | | 1.00E+00 | | 1.00E+00 |
| AT4G17950 | AQNTPEPASAPANM(ox)LSFGGVGGPGS(ph)PR | -0.69 | 6.09E-02 | -0.30 | 3.46E-01 | -0.24 | 4.38E-01 | 0.14 | 8.11E-01 |
| AT4G17950 | AQNTPEPASAPANMLSFGGVGGPGS(ph)PR | -0.13 | 9.40E-01 | -0.50 | 4.19E-02 | 1.15 | 8.09E-03 | 0.79 | 2.16E-01 |
| AT4G20400 | GESSLEPDSTPS(ph)SPK | 1.43 | 7.80E-03 | | 1.00E+00 | | 1.00E+00 | -0.54 | 9.17E-02 |
| AT4G21160 | TPAFLS(ph)SSLSK | | 1.00E+00 | | 1.00E+00 | -0.78 | 2.82E-01 | | 1.00E+00 |
| AT4G22750 | SES(ph)ETEPLQSL | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G24630 | YETTVSADGRQT(ph)PSVQIPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G28610 | TSS(ph)IPSTQKPSPVEDSFM(ox)R | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G29190 | NNPLFGFGS(ph)PR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G38900 | LSFGDESLKPPPS(ph)PGSM(ox)SR | | 6.92E-02 | | 1.00E+00 | | 1.00E+00 | | 5.05E-02 |
| AT4G39680 | ADMDAGKGKS(ph)PENK | | 1.00E+00 | -0.23 | 8.94E-01 | -1.36 | 2.02E-01 | | 1.00E+00 |
| AT4G39680 | LNLDRSS(ph)GDES(ph)MEDEPETK | 1.31 | 9.75E-02 | -0.11 | 6.40E-01 | -0.17 | 8.83E-01 | -1.58 | 7.90E-02 |
| AT4G39680 | NQTTPVTPVEAAFSTETTPVT(ph)AEKTPEPTQTK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G39680 | SDS(ph)SVS(ph)EDGPKER | 0.26 | 7.47E-01 | 0.80 | 1.65E-02 | -1.13 | 1.37E-01 | -0.58 | 2.17E-01 |
| AT4G39680 | SDS(ph)SVSEDGPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G39680 | VPEAQITNSAT(ph)PTT(ph)TPR | -0.51 | 6.03E-02 | 1.28 | 1.13E-01 | -1.70 | 1.00E+00 | 0.08 | 7.76E-01 |
| AT5G07350 | IGIWQYGDIES(ph)DDEDTGPAR | -0.22 | 8.40E-01 | -1.81 | 3.68E-04 | 1.85 | 2.28E-04 | 0.26 | 6.75E-01 |
| AT5G09850 | FNQPGDLEPPSLIADEDS(ph)PVQK | -0.33 | 7.66E-01 | -0.84 | 9.64E-02 | 1.40 | 3.47E-02 | 0.89 | 3.63E-01 |
| AT5G11260 | EGIES(ph)DEEIR | 0.02 | 9.61E-01 | 0.24 | 3.55E-01 | 0.11 | 7.36E-01 | 0.34 | 2.41E-01 |
| AT5G14270 | GLGTIDLEPM(ox)LDGATSAS(ph)PTR | -0.56 | 9.28E-02 | 0.37 | 9.92E-02 | -0.42 | 1.31E-01 | 0.51 | 7.33E-02 |
| AT5G14270 | GSSVGGLDQLESAS(ph)PEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G14540 | PLPQGLPM(ox)ASAISSGGSGGGSDS(ph)PR | -0.34 | 3.60E-01 | 0.42 | 1.88E-01 | -0.41 | 2.60E-01 | 0.36 | 2.86E-01 |
| AT5G14540 | SYGS(ph)M(ox)DSLEPSK | | 1.00E+00 | | 2.01E-01 | | 1.01E-02 | | 1.00E+00 |
| AT5G15020 | IEKEEGELS(ph)PVGDSEDNFVVYEDR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G18230 | NIM(ox)GVESNVQPLT(ph)SPLSK | | 1.00E+00 | -0.09 | 3.83E-01 | -0.05 | 4.84E-01 | | 1.00E+00 |
| AT5G18230 | VGIPNQPQPSQPPS(ph)PIPANGSR | -0.19 | 4.48E-01 | | 1.00E+00 | -0.73 | 2.89E-01 | | 1.00E+00 |
| AT5G19520 | AS(ph)PSFNPLAS(ph)PDSDAGIEK | | 1.00E+00 | 1.34 | 1.01E-02 | -0.57 | 1.39E-01 | | 1.00E+00 |
| AT5G19520 | ASPSFNPLAS(ph)PDSDAGIEK | 0.10 | 7.23E-01 | 0.74 | 3.19E-02 | -0.04 | 9.49E-01 | 0.59 | 1.37E-01 |
| AT5G19520 | EQFGAGS(ph)FAR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G22650 | GMDVDEDDS(ph)DDDEEEDS(ph)EDEEEEETPK | | 1.00E+00 | | 1.00E+00 | 0.27 | 1.00E+00 | | 1.00E+00 |
| AT5G22650 | GMDVDEDDS(ph)DDDEEEDS(ph)EDEEEEETPKKPEPINK | -0.02 | 8.43E-01 | 0.47 | 1.42E-01 | 0.30 | 3.59E-01 | 0.79 | 2.31E-02 |
| AT5G22650 | SPVNANQS(ph)PK | -0.64 | 2.21E-01 | 0.97 | 6.55E-02 | -0.63 | 2.25E-01 | 0.98 | 7.33E-02 |
| AT5G23750 | EVEEEKKEGS(ph)VNR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G24450 | LPVTS(ph)SPK | 1.43 | 1.52E-02 | 0.23 | 6.39E-01 | -0.30 | 5.03E-01 | -1.50 | 8.64E-03 |
| AT5G27650 | DGVVGS(ph)EEEDEIK | | 1.00E+00 | -0.03 | 1.00E+00 | 0.06 | 1.00E+00 | | 1.00E+00 |
| AT5G28040 | DTDFSAES(ph)PDLEEDGGGGGGGR | 0.68 | 4.32E-02 | 0.34 | 5.32E-01 | -0.06 | 9.64E-01 | -0.40 | 1.29E-01 |
| AT5G35330 | SSS(ph)PNEDRGENQLVVYDLK | -0.19 | 8.47E-01 | -0.60 | 2.51E-01 | 1.13 | 2.14E-01 | 0.72 | 1.89E-01 |
| AT5G40340 | KQS(ph)DGEEETQKEPSESTK | 0.39 | 2.99E-02 | 0.08 | 9.62E-01 | -0.58 | 1.53E-01 | -0.90 | 8.20E-03 |
| AT5G42520 | EM(ox)EPNDGLPTS(ph)PPAGSTLESAKPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G45420 | VGYGQILEPEQIHDES(ph)STDNERESR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G45420 | VGYGQILEPEQIHDESS(ph)TDNER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G46760 | SAANDS(ph)DHS(ph)DLEASVVK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G47430 | ILEAGNDS(ph)TENVGS(ph)VGHIPDLESAR | 0.06 | 3.18E-01 | 0.82 | 1.00E+00 | 0.28 | 9.57E-02 | 1.04 | 1.00E+00 |
| AT5G47430 | S(ph)PPVVVSDVSEDK | -0.88 | 1.00E+00 | 1.05 | 1.04E-01 | -2.28 | 1.00E+00 | -0.35 | 2.82E-01 |
| AT5G47430 | S(ph)PPVVVSDVSEDKLR | -0.03 | 8.35E-01 | -0.33 | 5.11E-02 | 0.43 | 3.58E-01 | 0.14 | 9.54E-01 |
| AT5G49400 | TKPSVDDLDGS(ph)DDDDEEERPDATNGK | | 1.00E+00 | 0.10 | 9.47E-01 | -0.04 | 8.80E-01 | | 1.00E+00 |
| AT5G54310 | ARS(ph)PPRVEQER | -0.38 | 8.14E-01 | -0.50 | 2.98E-01 | 0.10 | 3.20E-01 | -0.02 | 6.52E-01 |
| AT5G61150 | GIES(ph)DEEES(ph)PPR | 0.70 | 5.88E-02 | -0.57 | 1.27E-01 | 0.87 | 5.67E-02 | -0.41 | 1.92E-01 |
| AT5G61150 | GKDS(ph)EDEYEEDAEEDEEER | | 1.00E+00 | 0.01 | 4.55E-01 | -0.22 | 2.71E-01 | | 1.00E+00 |
| AT5G61150 | KAVIDDS(ph)DED | -0.13 | 8.04E-01 | -0.31 | 3.19E-01 | -0.36 | 7.48E-01 | -0.54 | 3.51E-01 |
| AT5G61150 | NVFGS(ph)S(ph)DDEDAEEYVR | -0.19 | 6.52E-01 | 0.50 | 3.82E-01 | 0.31 | 7.19E-01 | 1.00 | 1.50E-01 |
| AT5G61150 | S(ph)NRYSDEDEEEEEVAGGR | 1.00 | 1.00E+00 | -2.33 | 1.00E+00 | 3.13 | 1.00E+00 | -0.20 | 1.00E+00 |
| AT5G61150 | S(ph)PSEEKEEVQVAQSDVNIR | | 1.00E+00 | 0.15 | 2.72E-01 | -0.02 | 4.08E-01 | | 1.00E+00 |
| AT5G61780 | TGIWEYGDIQS(ph)DDEDNVPVR | -0.14 | 8.17E-01 | -1.52 | 8.55E-04 | 1.84 | 1.52E-04 | 0.46 | 2.82E-01 |
| AT5G61780 | TGIWEYGDIQS(ph)DDEDNVPVRKPGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 1.58 | 5.10E-02 |

signaling.

(Ca) calcium; (G) G-proteins; (S) sugar and nutrients; (L) light; (M) MAP kinases; (PI) phoshpoinositides; (RK) receptor kinases; (U) unspecified

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G01960 | (G) | NPPLS(ph)PQGGK | | 1.00E+00 | 0.28 | 1.00E+00 | | 1.00E+00 | 0.39 | 6.55E-02 |
| AT1G05150 | (Ca) | DNDVPVSYS(ph)GSGGPTK | -0.07 | 7.70E-01 | 0.51 | 7.13E-02 | -0.53 | 6.29E-02 | 0.04 | 8.73E-01 |
| AT1G06400 | (G) | S(ph)VDGGGESADLPGKGETINVK | 0.41 | 4.37E-01 | 1.11 | 8.88E-02 | 0.72 | 1.60E-01 | 1.42 | 3.66E-02 |
| AT1G06840 | (RK) | LAPVPDM(ox)EGIS(ph)PQHVSTVVK | -0.68 | 3.73E-01 | 0.00 | 9.58E-01 | -0.09 | 7.23E-01 | 0.59 | 6.55E-01 |
| AT1G07650 | (RK) | SLS(ph)FSTSGPR | 0.69 | 4.22E-02 | 0.25 | 6.27E-01 | -0.28 | 9.68E-01 | -0.72 | 4.89E-01 |
| AT1G09210 | (Ca) | DAPAES(ph)DAEDEPEDDEGGDDSDSESK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G09210 | (Ca) | KNEEEESKDAPAES(ph)DAEDEPEDDEGGDDSDSESK | | 1.00E+00 | 1.66 | 2.20E-01 | | 1.00E+00 | 0.34 | 4.09E-01 |
| AT1G09210 | (Ca) | NEEEESKDAPAES(ph)DAEDEPEDDEGGDDS(ph)DSESKAEETK | | 1.00E+00 | 1.39 | 2.20E-01 | | 1.00E+00 | 1.62 | 4.09E-01 |
| AT1G09210 | (Ca) | NEEEESKDAPAES(ph)DAEDEPEDDEGGDDSDSESK | 0.64 | 1.00E+00 | 1.77 | 2.20E-01 | -0.50 | 1.00E+00 | 0.63 | 4.09E-01 |
| AT1G10870 | (G) | SGS(ph)LTEGSLGYNTIDLR | 0.71 | 2.45E-01 | | 1.00E+00 | | 1.00E+00 | -0.14 | 8.06E-01 |
| AT1G10900 | (PI) | SAS(ph)VNVEELR | 0.74 | 7.48E-03 | 0.51 | 5.76E-02 | -0.43 | 1.44E-01 | -0.65 | 3.47E-02 |
| AT1G16670 | (RK) | S(ph)GIQFDWSSR | 1.45 | 2.14E-02 | 0.03 | 3.83E-01 | -0.12 | 3.10E-01 | -1.54 | 3.90E-02 |
| AT1G17340 | (PI) | SGS(ph)NLDIENM(ox)RPLIR | -0.68 | 1.90E-01 | | 1.00E+00 | | 1.00E+00 | 0.55 | 5.65E-01 |
| AT1G18210 | (Ca) | AMGTS(ph)YTETELNR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G18390 | (RK) | SGPLVAQS(ph)PDSVIVK | 0.99 | 3.54E-02 | 1.17 | 1.86E-02 | -0.15 | 5.88E-01 | 0.02 | 9.88E-01 |
| AT1G18840 | (Ca) | HEDVS(ph)DDEIQVSEVQPTDSQDVASVPDDSLSESEK | | 1.00E+00 | | 4.56E-02 | | 1.00E+00 | | 1.00E+00 |
| AT1G19870 | (Ca) | DDVLGEEGKTDIDS(ph)PDTTNTIK | 0.23 | 4.61E-01 | 0.23 | 1.37E-02 | -0.22 | 5.15E-01 | -0.23 | 1.00E+00 |
| AT1G19870 | (Ca) | ETLESALLKSPS(ph)PDNNNVSEK | -0.55 | 7.49E-01 | -0.32 | 2.98E-01 | -0.17 | 2.02E-01 | 0.05 | 8.06E-01 |
| AT1G19870 | (Ca) | FEELTSSTGS(ph)NK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G19870 | (Ca) | GTET(ph)EEDDLIGTELQGPSAADAAK | | 1.00E+00 | | 2.98E-01 | | 2.02E-01 | | 1.00E+00 |
| AT1G19870 | (Ca) | IEEDVTS(ph)EVEM(ox)ASK | 0.07 | 9.63E-01 | 0.81 | 1.75E-01 | -0.23 | 9.33E-01 | 0.51 | 2.61E-01 |
| AT1G19870 | (Ca) | SPS(ph)PDNNNVSEK | 0.24 | 6.20E-01 | 0.98 | 2.00E-01 | -0.38 | 8.12E-01 | 0.36 | 6.15E-01 |
| AT1G19870 | (Ca) | TRETLESALLKS(ph)PS(ph)PDNNNVSEK | -1.57 | 4.20E-02 | | 1.00E+00 | | 1.00E+00 | 1.46 | 1.56E-01 |
| AT1G19870 | (Ca) | VEPEES(ph)ESDDVIIVR | 0.78 | 1.73E-03 | 0.97 | 7.82E-04 | -0.31 | 3.07E-01 | -0.12 | 4.88E-01 |
| AT1G19870 | (Ca) | VEPEES(ph)ESDDVIIVRK | 0.06 | 4.75E-01 | -0.34 | 1.21E-01 | 0.63 | 1.33E-01 | 0.22 | 1.60E-01 |
| AT1G20760 | (Ca) | FDS(ph)FNTSEAGAGFSSQPER | 0.42 | 4.96E-01 | -0.85 | 1.00E+00 | 0.71 | 4.18E-01 | -0.56 | 1.00E+00 |
| AT1G20760 | (Ca) | FDS(ph)INSSK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G20760 | (Ca) | FDS(ph)INSSR | 0.09 | 3.94E-01 | 0.33 | 6.63E-01 | -0.38 | 4.78E-01 | -0.14 | 4.60E-01 |
| AT1G20760 | (Ca) | FDSINS(ph)SKDFGGPSLSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G20760 | (Ca) | FGNS(ph)PPR | 0.69 | 8.04E-02 | 0.07 | 9.46E-01 | 0.21 | 4.96E-01 | -0.41 | 2.39E-01 |
| AT1G20760 | (Ca) | NATEVPS(ph)PDYSQGK | 0.07 | 7.06E-01 | -0.09 | 7.81E-01 | 0.34 | 3.77E-01 | 0.18 | 7.53E-01 |
| AT1G20760 | (Ca) | VSSDES(ph)PTKR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G21630 | (Ca) | FDS(ph)FNSNNNDAFSLSR | -0.12 | 8.22E-01 | | 1.00E+00 | | 1.00E+00 | -0.37 | 1.97E-01 |
| AT1G21630 | (Ca) | FDS(ph)IGSTR | 0.66 | 1.01E-01 | -0.11 | 6.99E-01 | 0.09 | 7.09E-01 | -0.68 | 1.18E-01 |
| AT1G21630 | (Ca) | GIEADSS(ph)PR | -0.24 | 1.00E+00 | 0.33 | 8.14E-01 | 0.17 | 3.57E-01 | 0.74 | 5.59E-02 |
| AT1G21630 | (Ca) | KGIEADS(ph)SPR | -0.19 | 8.14E-01 | -0.02 | 7.38E-01 | -0.06 | 8.82E-01 | 0.11 | 9.52E-01 |
| AT1G21630 | (Ca) | YDS(ph)FNAQSYDSSSNNNASETPK | | 1.00E+00 | 0.48 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G25390 | (RK) | DNS(ph)KSDVEFSQVFFK | -0.63 | 3.11E-01 | -0.69 | 1.05E-02 | 0.89 | 2.56E-02 | 0.83 | 1.80E-01 |
| AT1G30440 | (L) | FSTPLGS(ph)GNVLSEEEQK | 1.32 | 2.84E-02 | -0.52 | 2.21E-01 | 0.77 | 2.46E-01 | -1.06 | 1.00E+00 |
| AT1G30440 | (L) | S(ph)GGYVGGPNEGGGGGGGWATAVR | | 2.84E-02 | | 2.21E-01 | | 1.00E+00 | | 1.00E+00 |
| AT1G31930 | (G) | GLNAVEGDS(ph)GGEEANDEGTVTTPQSVYTLNPR | -0.24 | 3.47E-01 | 0.09 | 2.00E-01 | 0.13 | 1.54E-02 | 0.46 | 4.55E-02 |
| AT1G49340 | (PI) | LIS(ph)GAFSQAPQPEDDSFNEM(ox)LIAR | -0.46 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.62 | 4.98E-02 |
| AT1G51805 | (RK) | VEGTLPSYM(ox)QASDGRS(ph)PR | -0.33 | 6.58E-02 | 1.26 | 1.00E-02 | -1.67 | 3.61E-03 | -0.08 | 4.74E-01 |
| AT1G51890 | (RK) | ESVEFS(ph)PSSASDFSPLAR | 0.05 | 6.84E-01 | -0.70 | 1.44E-01 | 1.04 | 4.62E-02 | 0.29 | 6.74E-01 |
| AT1G52380 | (G) | LAPAEAVVEDNQKAS(ph)DIEEGDEVDSK | -0.32 | 8.74E-01 | 0.21 | 7.29E-01 | 0.32 | 6.72E-01 | 0.85 | 3.24E-01 |
| AT1G53210 | (Ca) | QWLIQAM(ox)GGAPSGPEAGPRT(ph)M(ox)K | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G53430 | (RK) | LNDDENTHIS(ph)TR | 0.23 | 5.45E-01 | -0.05 | 8.96E-01 | -0.04 | 9.76E-01 | -0.32 | 5.39E-01 |
| AT1G53440 | (RK) | LLDDLT(ph)DVEIE | 0.63 | 5.89E-02 | | 1.00E+00 | | 1.00E+00 | 1.16 | 1.36E-02 |
| AT1G53730 | (RK) | NKS(ph)FDDEDSTR | 1.40 | 3.07E-02 | 0.92 | 1.00E+00 | -0.83 | 5.84E-02 | -1.32 | 1.00E+00 |
| AT1G55040 | (G) | SSFASDEFS(ph)EDEKFPESK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G56340 | (Ca) | DAPAES(ph)DAEEEAEDDDNEGDDS(ph)DNESKSEETK | | 1.00E+00 | 0.28 | 2.50E-01 | 0.16 | 1.00E+00 | | 1.00E+00 |
| AT1G56340 | (Ca) | DAPAES(ph)DAEEEAEDDDNEGDDSDNESK | 0.07 | 3.80E-01 | 1.33 | 1.99E-01 | -0.47 | 4.31E-01 | 0.78 | 4.18E-03 |
| AT1G56340 | (Ca) | EEEESKDAPAES(ph)DAEEEAEDDDNEGDDS(ph)DNESKSEETK | -2.13 | 2.48E-02 | 0.65 | 4.42E-02 | -1.70 | 1.55E-02 | 1.09 | 1.36E-01 |
| AT1G56340 | (Ca) | EEEESKDAPAES(ph)DAEEEAEDDDNEGDDSDNESK | -1.94 | 1.00E+00 | 1.04 | 1.36E-01 | -2.16 | 8.53E-02 | 0.82 | 1.70E-02 |
| AT1G56340 | (Ca) | REEEESKDAPAES(ph)DAEEEAEDDDNEGDDSDNESK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G60890 | (PI) | SLS(ph)ELTTTSGLTR | 0.54 | 8.76E-02 | 0.71 | 2.37E-02 | -0.19 | 3.94E-01 | -0.02 | 9.53E-01 |
| AT1G66880 | (RK) | SLPITS(ph)YSSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G67310 | (L) | GFRQDVESTEDS(ph)EDEDILK | -1.82 | 1.79E-01 | | 1.00E+00 | | 1.00E+00 | 1.34 | 1.84E-01 |
| AT1G67310 | (L) | QDVESTEDS(ph)EDEDILK | 0.56 | 1.17E-01 | 0.23 | 4.83E-01 | -0.13 | 8.01E-01 | -0.46 | 2.45E-01 |
| AT1G71010 | (PI) | VQS(ph)FDSAIR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G74690 | (Ca) | SGGM(ox)LETQNVGPEEIS(ph)DDEIELPEGK | -0.49 | 4.20E-01 | -0.70 | 1.15E-01 | 0.57 | 1.54E-01 | 0.37 | 5.44E-01 |
| AT2G03150 | (G) | DAEKKS(ph)PGDTSGTPTTGTK | 1.38 | 1.16E-02 | -0.30 | 4.80E-01 | 0.23 | 2.19E-01 | -1.45 | 1.08E-01 |
| AT2G03150 | (G) | SGYVPTLPDS(ph)PK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G03150 | (G) | TVDVKQET(ph)GSPDTK | 0.44 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -1.08 | 1.95E-02 |
| AT2G17290 | (Ca) | NSLNIS(ph)M(ox)R | -0.19 | 7.13E-01 | 0.44 | 2.57E-01 | -1.03 | 7.86E-02 | -0.41 | 6.04E-01 |
| AT2G17290 | (Ca) | NSLNIS(ph)M(ox)RDV | 0.82 | 1.53E-01 | 0.27 | 7.85E-01 | -0.49 | 6.76E-01 | -1.03 | 1.21E-01 |
| AT2G17290 | (Ca) | STTTTVSSVHS(ph)PTTDQDFSK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G21150 | (L) | LSFAEDFENGS(ph)DEDDGENK | 0.45 | 4.92E-01 | 0.20 | 5.09E-01 | 0.61 | 4.22E-01 | 0.37 | 3.35E-01 |
| AT2G21150 | (L) | LSFAEDFENGS(ph)DEDDGENKSSGTGNLR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.63 | 3.75E-01 |
| AT2G21880 | (G) | GQYHDS(ph)VTDIIDPDQSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G23770 | (RK) | KPPM(ox)S(ph)DQEFDPLDGLSGM(ox)VVESLK | | 1.00E+00 | | 1.00E+00 | | 3.16E-01 | | 1.00E+00 |
| AT2G23770 | (RK) | TQTQEETGNLDS(ph)FM(ox)GK | -0.52 | 5.38E-01 | 0.21 | 7.73E-01 | -0.81 | 2.80E-01 | -0.08 | 8.90E-01 |
| AT2G27060 | (RK) | IQNS(ph)PDNPTSR | 1.18 | 4.71E-02 | | 1.00E+00 | | 1.00E+00 | -1.30 | 4.88E-02 |
| AT2G31680 | (G) | (ac)S(ph)SDDEGGEEYLFK | 0.52 | 1.66E-01 | 1.12 | 7.72E-03 | -0.40 | 2.66E-01 | 0.20 | 5.22E-01 |
| AT2G32450 | (Ca) | DNNVPVSYS(ph)GNGIPTK | -0.47 | 3.00E-01 | 0.90 | 1.32E-01 | -1.01 | 6.67E-02 | 0.36 | 5.44E-01 |
| AT2G33990 | (Ca) | TVS(ph)LSSVPAK | | 1.00E+00 | | 9.07E-02 | | 8.63E-02 | | 1.00E+00 |
| AT2G37050 | (RK) | GGIS(ph)DEFSR | 0.15 | 3.02E-01 | 0.55 | 8.42E-03 | -0.81 | 7.18E-02 | -0.41 | 1.48E-02 |
| AT2G43130 | (G) | (ac)S(ph)DDDERGEEYLFK | 0.80 | 9.07E-03 | 0.50 | 1.68E-01 | 0.02 | 7.61E-01 | -0.28 | 2.28E-01 |
| AT2G43680 | (Ca) | GPGS(ph)PGGVVLEK | | 1.00E+00 | 0.00 | 9.98E-01 | -0.57 | 9.62E-02 | | 1.00E+00 |
| AT2G43680 | (Ca) | LDAPRPTT(ph)PKPPS(ph)PR | | 1.00E+00 | 0.53 | 3.76E-01 | -0.34 | 4.23E-01 | | 1.00E+00 |
| AT2G43680 | (Ca) | VAS(ph)PRPT(ph)SPR | 1.07 | 1.49E-02 | -0.19 | 6.84E-01 | 0.02 | 7.47E-01 | -1.24 | 1.25E-02 |
| AT2G45340 | (RK) | SDES(ph)EFLK | -0.95 | 2.92E-01 | 1.47 | 3.91E-02 | -1.25 | 7.01E-02 | 1.17 | 1.79E-01 |
| AT2G46700 | (Ca) | TES(ph)GIFR | 0.51 | 5.70E-02 | 0.50 | 5.10E-02 | -0.31 | 1.83E-01 | -0.32 | 1.81E-01 |
| AT2G48010 | (RK) | LDS(ph)MSESTTLVK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G02880 | (RK) | LIEEVSHSSGSPNPVS(ph)D | 0.96 | 1.50E-02 | 0.76 | 5.81E-02 | -0.20 | 3.99E-01 | -0.40 | 2.14E-01 |
| AT3G08510 | (PI) | EVPS(ph)FIQR | 1.13 | 6.38E-02 | 1.10 | 1.90E-01 | -0.95 | 2.37E-01 | -0.99 | 8.12E-02 |
| AT3G08680 | (RK) | ASS(ph)PEM(ox)IR | -0.19 | 4.57E-01 | | 1.00E+00 | | 1.00E+00 | 0.02 | 7.60E-01 |
| AT3G08680 | (RK) | ASS(ph)PEMIR | 1.11 | 4.54E-05 | -0.07 | 5.16E-01 | 0.44 | 6.09E-03 | -0.74 | 4.28E-04 |
| AT3G13460 | (Ca) | LSLDS(ph)PAK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G13530 | (M) | TPS(ph)SVSGNELAR | | 1.00E+00 | 0.83 | 1.18E-01 | -0.70 | 1.30E-01 | | 1.00E+00 |
| AT3G13530 | (M) | VRS(ph)GQLDPNNPIFGQNETSSLSM(ox)IDQPDVLK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G13690 | (RK) | LNLVGS(ph)PK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G14205 | (PI) | S(ph)FETIPESR | 1.20 | 1.76E-02 | 1.06 | 1.21E-02 | 0.04 | 9.94E-01 | -0.10 | 9.40E-01 |
| AT3G14205 | (PI) | SLS(ph)ESSIISESSPAALGPVGR | 0.08 | 7.52E-01 | 0.61 | 1.59E-01 | 0.78 | 2.08E-01 | 1.31 | 4.68E-03 |
| AT3G14350 | (RK) | KLDTSLS(ph)M(ox)NLRPPPSER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G14590 | (Ca) | STASTS(ph)PNNESSSTDKNQEGK | | 1.00E+00 | -0.19 | 2.78E-01 | -0.07 | 2.84E-01 | | 1.00E+00 |
| AT3G14840 | (RK) | LDEEENTHIS(ph)TR | 0.57 | 1.55E-01 | | 1.00E+00 | | 1.00E+00 | 0.28 | 3.13E-01 |
| AT3G14840 | (RK) | LLEASVNNEKDEES(ph)VR | | 1.55E-01 | | 1.00E+00 | | 1.00E+00 | | 3.13E-01 |
| AT3G14840 | (RK) | NLDFQIS(ph)SFSLR | | 1.55E-01 | | 1.00E+00 | | 1.00E+00 | | 3.13E-01 |
| AT3G17840 | (RK) | SYVNEYS(ph)PSAVK | 0.36 | 4.28E-01 | 0.08 | 9.69E-01 | -0.30 | 5.30E-01 | -0.57 | 2.05E-01 |
| AT3G20410 | (Ca) | AAAAAPGLS(ph)PK | 0.64 | 3.44E-01 | 0.58 | 3.00E-01 | -0.02 | 9.57E-01 | -0.08 | 8.69E-01 |
| AT3G20410 | (Ca) | EVLS(ph)DVDSDNDGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G20410 | (Ca) | SNS(ph)ILENAFEDVK | | 1.00E+00 | -0.01 | 1.00E+00 | | 1.00E+00 | -0.26 | 3.33E-01 |
| AT3G20410 | (Ca) | SVEVGVTNQDPPSYT(ph)PQAR | | 1.00E+00 | | 1.00E+00 | -0.28 | 4.32E-01 | | 1.00E+00 |
| AT3G22170 | (L) | SLPDVVTS(ph)PTQGLISVEEDNHSR | -0.94 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 1.43 | 1.00E+00 |
| AT3G22190 | (Ca) | KNS(ph)VDFDFEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G22380 | (L) | AGS(ph)FRDSPEEEGPVELPEAAR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.60 | 3.65E-01 |
| AT3G22380 | (L) | QISSTS(ph)PANR | -1.13 | 8.73E-02 | | 1.00E+00 | -1.24 | 1.00E+00 | | 8.21E-02 |
| AT3G22380 | (L) | VS(ph)SPISNPQTLPQSSITLAANSSSSNVSAIAPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.65 | 3.40E-01 |
| AT3G22380 | (L) | VSPASILASPSPPAPTS(ph)PSSSSISVR | -0.09 | 6.72E-01 | -0.14 | 2.68E-01 | 0.06 | 3.73E-01 | 0.01 | 6.07E-01 |
| AT3G24660 | (RK) | KSSIES(ph)EDDLEEGDEEDEIGEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G24660 | (RK) | SSIES(ph)EDDLEEGDEEDEIGEK | -0.28 | 5.15E-01 | 1.04 | 1.75E-02 | -0.82 | 4.47E-02 | 0.50 | 2.60E-01 |
| AT3G24660 | (RK) | SSIES(ph)EDDLEEGDEEDEIGEKEGGEGK | -0.29 | 4.01E-01 | -0.62 | 3.86E-02 | 0.42 | 2.83E-01 | 0.09 | 7.65E-01 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT3G28450 | (RK) | S(ph)GLTEVGVSGLAQR | 0.37 | 1.71E-01 | 0.22 | 4.59E-01 | -0.19 | 5.25E-01 | -0.34 | 2.03E-01 |
| AT3G43300 | (G) | NANEDSAS(ph)TGEPIETK | 0.85 | 2.46E-01 | -0.63 | 8.29E-02 | 0.99 | 1.88E-01 | -0.49 | 4.14E-01 |
| AT3G51550 | (RK) | SLAS(ph)EDSDGLTPSAVFSQIM(ox)NPK | 0.20 | | -2.04 | | 2.60 | 0.37 | | |
| AT3G52870 | (Ca) | TLS(ph)GGLGS(ph)PK | 0.22 | 2.53E-01 | 0.44 | 2.81E-02 | -0.65 | 4.12E-03 | -0.43 | 3.99E-02 |
| AT3G55020 | (G) | GASDIDS(ph)EDEFYDVER | 0.45 | 2.79E-01 | 0.39 | 3.87E-01 | 0.08 | 6.41E-01 | 0.01 | 9.32E-01 |
| AT3G56760 | (Ca) | SES(ph)GIFR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G57530 | (Ca) | EGFQIM(ox)DT(ph)SQR | | 1.00E+00 | 0.47 | 1.67E-01 | 0.64 | 1.20E-01 | | 1.00E+00 |
| AT3G59770 | (PI) | LGSSSS(ph)VSLDK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G61050 | (Ca) | SGSST(ph)PVNTVPENDGAK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G04700 | (Ca) | LGS(ph)NLSK | | 3.12E-01 | 0.96 | 1.00E+00 | -1.56 | 1.00E+00 | | 6.51E-02 |
| AT4G04700 | (Ca) | TES(ph)SLQPEGELLPIIN | 1.69 | 2.67E-01 | 1.32 | 4.07E-01 | 1.28 | 3.40E-01 | 0.92 | 5.01E-01 |
| AT4G08500 | (M) | S(ph)LEFPEPTSFR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G08850 | (RK) | GEHPGDLVSTLSS(ph)SPPDATLSLK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G08850 | (RK) | QIEEHT(ph)DSESGGETLSIFSFDGK | -2.37 | 1.15E-01 | | 1.00E+00 | | 1.00E+00 | -0.45 | 2.01E-01 |
| AT4G12640 | (G) | LGS(ph)SEGYLQDR | | 1.00E+00 | -1.10 | 1.00E+00 | 1.22 | 5.52E-02 | | 1.00E+00 |
| AT4G13350 | (G) | DLGSAS(ph)PPVAR | 1.20 | 1.39E-01 | 1.16 | 1.12E-01 | -0.07 | 7.68E-01 | -0.11 | 8.84E-01 |
| AT4G13350 | (G) | DLGSAS(ph)PPVARPVR | 1.50 | 1.07E-01 | 0.51 | 7.28E-01 | 0.36 | 2.45E-01 | -0.63 | 2.92E-01 |
| AT4G13350 | (G) | KTSEEGSQS(ph)PEQVK | 2.03 | 2.09E-05 | -0.36 | 5.34E-01 | 0.03 | 7.96E-01 | -2.36 | 2.62E-03 |
| AT4G13350 | (G) | S(ph)PGFETGSR | 0.92 | 1.14E-01 | -0.02 | 9.29E-01 | 0.38 | 4.67E-01 | -0.56 | 2.87E-01 |
| AT4G13350 | (G) | SM(ox)S(ph)APSLQPLQGVPSGGLQSSEVKPSGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G13350 | (G) | SS(ph)PGGRS(ph)PGFETGSR | 0.81 | 8.96E-02 | 0.50 | 1.00E+00 | -0.25 | 1.78E-01 | -0.56 | 1.11E-01 |
| AT4G13350 | (G) | TSEEGSQS(ph)PEQVK | 0.86 | 2.82E-01 | 0.35 | 9.82E-01 | -0.50 | 8.19E-01 | -1.00 | 2.43E-01 |
| AT4G13350 | (G) | TSEEGSQS(ph)PEQVKDLGS(ph)ASPPVARPVR | 0.34 | 1.93E-01 | | 1.00E+00 | | 1.00E+00 | 0.02 | 9.20E-01 |
| AT4G20940 | (RK) | LAVATGFS(ph)PSK | -0.16 | 1.46E-02 | 0.52 | 1.21E-02 | -0.44 | 2.35E-02 | 0.24 | 1.07E-03 |
| AT4G23650 | (Ca) | RGS(ph)SGSGTVGSSGSGTGGSR | -3.07 | 2.69E-01 | 1.33 | 2.30E-01 | -1.41 | 3.57E-01 | 2.98 | 1.78E-01 |
| AT4G27300 | (RK) | NVPDISS(ph)SLSLR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G29900 | (Ca) | (ac)SGQFNNS(ph)PR | 0.61 | 2.65E-03 | 0.99 | 9.71E-02 | -0.48 | 3.69E-01 | -0.10 | 4.47E-01 |
| AT4G29900 | (Ca) | (ac)SGQFNNS(ph)PRGEDKDVEAGTSSFTEYEDSPFDIASTK | -0.59 | 4.15E-01 | | 1.00E+00 | | 1.00E+00 | -0.28 | 6.34E-01 |
| AT4G29900 | (Ca) | GEDKDVEAGTSS(ph)FTEYEDSPFDIASTK | -2.28 | 4.15E-01 | | 1.00E+00 | | 1.00E+00 | 1.87 | 1.00E+00 |
| AT4G29900 | (Ca) | QAALVLNAS(ph)R | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G29900 | (Ca) | VTGIAS(ph)PLPTPGGGDFGIGQEQIVSISR | -1.47 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 1.13 | 9.80E-02 |
| AT4G33240 | (PI) | SES(ph)M(ox)VVK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 3.30E-01 |
| AT4G35310 | (Ca) | NSLNIS(ph)MRDA | | 1.00E+00 | 0.07 | 9.97E-01 | 0.62 | 1.59E-01 | | 1.00E+00 |
| AT4G35470 | (RK) | SDS(ph)QSSLNFSER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G01560 | (RK) | ISS(ph)TSLISGR | 0.50 | 1.42E-01 | 0.09 | 8.63E-01 | -0.15 | 6.83E-01 | -0.57 | 1.01E-01 |
| AT5G01950 | (RK) | VELASSSSVLSTSS(ph)SNVTR | 0.03 | 9.92E-01 | 0.13 | 7.19E-01 | -0.42 | 4.40E-01 | -0.31 | 7.00E-01 |
| AT5G03040 | (Ca) | DLS(ph)PPSTADAVNVTATDVPVVPSSSAPGVVR | -0.73 | 1.54E-01 | -1.06 | 1.00E+00 | 1.42 | 1.00E+00 | 1.10 | 1.06E-01 |
| AT5G03040 | (Ca) | LKPQS(ph)PLGGTTQENEGFTDK | 0.15 | 6.27E-01 | | 1.00E+00 | | 1.00E+00 | -0.13 | 3.69E-01 |
| AT5G03040 | (Ca) | NRDLS(ph)PPSTADAVNVTATDVPVVPSSSAPGVVR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 3.69E-01 |
| AT5G03040 | (Ca) | NSFFSPPT(ph)PSR | | 1.00E+00 | 0.83 | 8.00E-02 | -0.80 | 8.27E-02 | | 1.00E+00 |
| AT5G03040 | (Ca) | QSSSS(ph)PPPALAPR | -0.01 | 9.93E-01 | 0.02 | 7.94E-01 | 0.05 | 8.66E-01 | 0.09 | 6.74E-01 |
| AT5G04870 | (Ca) | DGDDSASM(ox)SNGDIASEAVS(ph)GELR | -1.27 | 1.57E-01 | | 1.00E+00 | -1.09 | 1.58E-01 | | 1.00E+00 |
| AT5G07300 | (Ca) | EMEVLDGDKGEKLES(ph)SSGR | | 1.00E+00 | 0.06 | 3.89E-01 | 0.26 | 2.78E-01 | | 1.00E+00 |
| AT5G10020 | (RK) | FSDQPVM(ox)LDVYS(ph)PDR | -0.37 | 3.17E-01 | | 1.00E+00 | -0.87 | 8.32E-02 | | 1.00E+00 |
| AT5G12080 | (U) | SVGS(ph)PAPVTPSK | 0.91 | 1.58E-03 | 0.13 | 2.74E-01 | 0.17 | 3.95E-02 | -0.61 | 6.31E-02 |
| AT5G12480 | (Ca) | DGS(ph)LQLEGET | | 1.00E+00 | 1.70 | 8.80E-02 | -0.82 | 3.50E-01 | | 1.00E+00 |
| AT5G16590 | (RK) | AS(ph)AEVLGK | 0.22 | 7.11E-01 | 0.80 | 2.38E-01 | -1.10 | 1.08E-01 | -0.51 | 3.86E-01 |
| AT5G16590 | (RK) | SPASPGPLS(ph)D | 0.80 | 1.49E-03 | 0.82 | 1.20E-03 | -0.59 | 6.56E-03 | -0.57 | 8.58E-03 |
| AT5G16590 | (RK) | WVSSITEQQS(ph)PSDVFDPELTR | -0.56 | 5.72E-01 | -1.51 | 3.01E-02 | 1.09 | 6.41E-02 | 0.14 | 9.72E-01 |
| AT5G19320 | (G) | ELISEDSVS(ph)PR | | 1.00E+00 | 0.47 | 2.35E-01 | | 1.00E+00 | | 1.00E+00 |
| AT5G19450 | (Ca) | EGS(ph)LQLEGEN | | 1.00E+00 | 1.17 | 3.06E-02 | -0.29 | 5.94E-01 | | 1.00E+00 |
| AT5G19450 | (Ca) | SNPFYSEAYTTNGS(ph)GTGFK | 0.36 | 5.43E-02 | -0.04 | 8.34E-01 | 0.19 | 2.92E-01 | -0.20 | 2.13E-01 |
| AT5G24430 | (Ca) | TES(ph)AIFR | | 1.00E+00 | 0.91 | 2.55E-02 | -0.36 | 1.62E-01 | | 1.00E+00 |
| AT5G25930 | (RK) | TATEAYEAPLLVSLS(ph)GRR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G44460 | (Ca) | SPS(ph)LNALR | -0.14 | 9.18E-01 | 0.87 | 7.71E-01 | -0.60 | 9.94E-01 | 0.42 | 7.24E-01 |
| AT5G46070 | (G) | GGKDS(ph)PADSASPSPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G49760 | (RK) | EIENIMQLAGLNPNSDS(ph)ATSSR | -0.54 | 4.00E-01 | -0.83 | 1.84E-01 | 1.02 | 5.92E-02 | 0.73 | 2.09E-01 |
| AT5G49760 | (RK) | GSGDPYGS(ph)ESFQYSGNFPASK | 0.39 | 2.51E-01 | | 1.00E+00 | | 1.00E+00 | 0.69 | 1.08E-01 |
| AT5G49770 | (RK) | LVGLNPNADS(ph)ATYEEASGDPYGR | 0.27 | 9.60E-01 | -0.09 | 8.14E-01 | 0.22 | 9.06E-01 | -0.14 | 7.56E-01 |
| AT5G52580 | (G) | T(ph)LSSLELPSSLPVASGQSVYPLDGGSSSENQR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G55850 | (S) | TGGKPGS(ph)PGKSSEGHVK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 3.88E-02 |
| AT5G56040 | (RK) | NLT(ph)SANVIGTGSSGVVYR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G58300 | (RK) | SPVQS(ph)PSRDDM(ox)VDLPR | 0.64 | 2.16E-01 | 0.42 | 4.42E-01 | -0.36 | 3.42E-01 | -0.58 | 2.31E-01 |
| AT5G58300 | (RK) | VSDSETTRPS(ph)SDDNSKPK | | 1.00E+00 | -2.78 | 5.07E-02 | 2.31 | 1.00E+00 | | 1.00E+00 |
| AT5G58350 | (M) | NRS(ph)LVDVQR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G58670 | (PI) | EYLQT(ph)QISK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G61790 | (Ca) | S(ph)GDEAEKKEETAAPR | -0.49 | 7.85E-01 | -0.29 | 8.28E-01 | -0.23 | 8.57E-01 | -0.04 | 9.35E-01 |
| AT5G62390 | (Ca) | EIAEGVTQIVQM(ox)LET(ph)EEE | -0.82 | 2.81E-01 | | 1.00E+00 | | 1.00E+00 | -0.03 | 8.59E-01 |
| AT5G63270 | (S) | ASGAGPNSLVS(ph)PQR | 0.58 | 2.57E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G64070 | (PI) | VDDGNES(ph)EGDESPEFSLFK | 0.45 | 3.19E-01 | -0.67 | 1.49E-01 | 1.01 | 3.50E-02 | -0.10 | 9.03E-01 |
| AT5G64070 | (PI) | VDDGNES(ph)EGDESPEFSLFKR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G64330 | (L) | APSPSTTNFAGS(ph)SPR | -0.16 | 1.62E-04 | 0.49 | 1.00E+00 | -0.39 | 1.00E+00 | 0.26 | 9.75E-05 |
| AT5G64330 | (L) | LLEHFLVQEQTEGS(ph)SPSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G64330 | (L) | LLEHFLVQEQTEGSS(ph)PSR | -2.21 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.57 | 1.97E-01 |
| AT5G64813 | (G) | EGTKGS(ph)SGNLVDAAR | 0.34 | 1.49E-01 | | 1.00E+00 | 0.89 | 2.98E-02 | | 1.00E+00 |
| AT5G64813 | (G) | LDEITS(ph)DDDQFYK | | 1.49E-01 | | 1.00E+00 | 2.65 | 2.98E-02 | | 1.00E+00 |
| AT5G64813 | (G) | LDEITS(ph)DDDQFYKR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G65700 | (RK) | DQPMTESAPESELS(ph)PK | 0.54 | 2.06E-02 | | 1.00E+00 | -0.35 | 4.50E-01 | | 1.00E+00 |
| AT5G65700 | (RK) | SGVQS(ph)PPDLLNL | -0.06 | 7.94E-01 | 0.53 | 1.83E-02 | -0.55 | 1.79E-02 | 0.04 | 8.06E-01 |
| AT5G66560 | (L) | GNIS(ph)ATDLQLIPGGAAK | | 1.03E-02 | | 1.00E+00 | | 1.00E+00 | | 2.56E-02 |

## stress.

(U) unspecified; (a) abiotic; (b) biotic

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G11310 | (b) | SVENYPSS(ph)PS(ph)PR | 0.18 | 6.70E-01 | 0.56 | 2.72E-01 | -0.80 | 1.20E-01 | -0.42 | 3.47E-01 |
| AT1G11360 | (a) | KSPTVVTVQPS(ph)SPRFPISTPTAGAQR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G11360 | (a) | SPTVVTVQPS(ph)SPR | 0.57 | 3.56E-01 | 1.85 | 2.13E-02 | -1.35 | 1.11E-01 | -0.07 | 9.00E-01 |
| AT1G11360 | (a) | SPTVVTVQPS(ph)SPRFPISTPTAGAQR | -0.94 | 7.22E-01 | | 1.00E+00 | | 1.00E+00 | 0.41 | 4.89E-01 |
| AT1G20440 | (a) | SNS(ph)SSSSSSDEEGEEKKEK | -2.33 | 1.00E+00 | -0.10 | 5.84E-01 | -1.00 | 3.62E-01 | 1.23 | 1.76E-02 |
| AT1G50570 | (a) | M(ox)KLPLDIDSPTQSENSSSSQQT(ph)PK | 0.60 | 1.64E-01 | 0.09 | 1.00E+00 | 0.40 | 1.00E+00 | -0.11 | 3.71E-01 |
| AT1G61560 | (b) | S(ph)RSVDESFANSFSPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G61560 | (b) | S(ph)VDESFANSFSPR | 0.68 | 1.89E-01 | 0.74 | 9.60E-02 | 0.04 | 8.61E-01 | 0.10 | 6.81E-01 |
| AT1G65280 | (a) | GLADENGLGSNS(ph)ADDVTGPK | -0.19 | 1.00E+00 | | 1.60E-01 | | 1.43E-01 | | 1.00E+00 |
| AT1G69450 | (a) | NDSLTSPLLS(ph)FSEV | 0.13 | 7.37E-01 | 0.39 | 4.79E-01 | 0.60 | 1.23E-01 | 0.85 | 4.43E-02 |
| AT1G74250 | (a) | AHEGEDEGAGLS(ph)ELEEEDDDAK | 1.78 | 1.00E+00 | | 1.00E+00 | 1.07 | 1.00E+00 | | 1.00E+00 |
| AT1G74250 | (a) | AHEGEDEGAGLS(ph)ELEEEDDDAKRK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G76180 | (a) | SDSSSSSSSEEEGS(ph)DGEKR | | 1.00E+00 | 1.37 | 1.48E-01 | -1.23 | 9.27E-02 | | 1.00E+00 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT2G17480 | (b) | LGGDGSAS(ph)PTASTVR | -0.30 | 5.38E-01 | 0.86 | 9.01E-02 | -0.74 | 1.71E-01 | 0.42 | 2.86E-01 |
| AT2G26890 | (a) | ILEISLNNVS(ph)SDDLNR | -0.63 | 1.15E-01 | -1.53 | 1.36E-03 | 1.23 | 5.88E-03 | 0.33 | 3.47E-01 |
| AT2G32120 | (a) | (ac)AEAAYTVAS(ph)DSENTGEEK | | 1.00E+00 | | 2.61E-01 | | 4.85E-01 | | 1.00E+00 |
| AT2G39200 | (b) | SLDQQTSFTASPS(ph)PPR | 0.80 | 1.55E-02 | 0.33 | 2.99E-01 | -0.02 | 7.61E-01 | -0.50 | 1.59E-01 |
| AT3G06340 | (a) | VSYNENLS(ph)DDDVDLVNDNGEGSGK | | 2.55E-01 | | 1.00E+00 | | 1.00E+00 | -0.05 | 1.00E+00 |
| AT3G10980 | (U) | FGLGSS(ph)PK | -0.34 | 1.61E-01 | 0.62 | 6.02E-02 | -0.62 | 5.65E-02 | 0.34 | 9.88E-02 |
| AT3G10980 | (U) | NISGASS(ph)PS(ph)PSR | | 1.00E+00 | 0.81 | 2.04E-02 | -1.11 | 1.13E-01 | | 1.00E+00 |
| AT3G10980 | (U) | NISGASSPS(ph)PSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G10980 | (U) | NM(ox)VS(ph)PNLVSPLPR | 0.93 | 1.17E-02 | 1.24 | 8.66E-03 | -1.14 | 1.18E-02 | -0.83 | 2.03E-02 |
| AT3G18690 | (b) | LASTENAS(ph)PR | -0.42 | 7.50E-01 | | 1.00E+00 | | 1.00E+00 | 0.87 | 4.54E-01 |
| AT3G25070 | (b) | ADES(ph)PEKVTVVPK | -0.06 | 4.53E-01 | 0.73 | 2.74E-01 | -0.93 | 1.61E-01 | -0.15 | 2.80E-01 |
| AT3G25070 | (b) | ASQNNSYDNKS(ph)PLHK | 0.26 | 3.54E-01 | 0.08 | 9.13E-01 | -0.19 | 8.73E-01 | -0.37 | 3.27E-01 |
| AT3G25070 | (b) | SSGANVSGS(ph)SRTPTHQSSR | 0.17 | 4.17E-01 | -0.49 | 4.93E-01 | 0.49 | 4.27E-01 | -0.17 | 4.94E-01 |
| AT4G19040 | (b) | NSLLM(ox)DEDS(ph)DDDDEFQIAESEQEPETSKPETDVK | -0.46 | 2.50E-01 | | 1.00E+00 | 1.48 | 6.56E-02 | | 1.00E+00 |
| AT4G23630 | (b) | IHHGGDSSSSSSSS(ph)DDEDEK | | 1.00E+00 | | 1.00E+00 | -0.70 | 8.80E-01 | | 1.00E+00 |
| AT4G23630 | (b) | KPSS(ph)PSSSMK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G27320 | (a) | IHHPS(ph)SPR | -1.16 | 3.62E-01 | 0.65 | 2.19E-01 | -0.75 | 3.65E-01 | 1.06 | 2.36E-01 |
| AT4G27320 | (a) | S(ph)RRDDDDDDDEDHEAK | | 1.00E+00 | -2.58 | 1.89E-02 | 0.92 | 4.19E-02 | | 1.00E+00 |
| AT5G45490 | (b) | LLAERDPVFVDDEVGPVGS(ph)THGQTDSSNR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G47910 | (b) | DIINNM(ox)KGPDRDS(ph)DIENNNSNNNSK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G47910 | (b) | GANSDTNS(ph)DTESIASDR | -0.30 | 9.12E-02 | | 1.00E+00 | | 1.00E+00 | -0.71 | 1.68E-01 |
| AT5G47910 | (b) | GNS(ph)SNDHELGILR | | 9.12E-02 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G47910 | (b) | GPDRDS(ph)DIENNNSNNNSK | 1.00 | 4.98E-02 | 0.25 | 5.93E-01 | 0.23 | 5.57E-01 | -0.52 | 2.30E-01 |
| AT5G47910 | (b) | ILSQM(ox)LS(ph)QK | 0.18 | 7.33E-01 | 0.23 | 3.37E-01 | -0.41 | 4.61E-02 | -0.36 | 7.19E-01 |
| AT5G48620 | (b) | SLS(ph)LQER | 0.37 | 2.15E-02 | 0.97 | 8.87E-03 | -1.23 | 4.70E-03 | -0.63 | 9.73E-02 |
| AT5G52640 | (a) | EIS(ph)DDEDEDEPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G54430 | (a) | IHHPPS(ph)PR | -1.79 | 9.39E-02 | 0.78 | 3.27E-01 | -0.55 | 4.99E-01 | 2.03 | 5.99E-02 |
| AT5G55530 | (a) | (ac)M(ox)DS(ph)PQSVVSPFK | -0.47 | 6.13E-02 | 0.68 | 9.83E-03 | -0.63 | 1.22E-02 | 0.52 | 4.97E-02 |
| AT5G55530 | (a) | SENSSSDSQKLPT(ph)PK | 1.03 | 1.62E-01 | -0.29 | 8.31E-01 | 0.65 | 3.52E-01 | -0.68 | 4.25E-01 |
| AT5G56030 | (a) | EIS(ph)DDEEEEEK | 1.21 | 4.06E-01 | 1.32 | 2.60E-01 | 0.08 | 7.64E-01 | 0.19 | 9.48E-01 |
| AT5G56030 | (a) | EIS(ph)DDEEEEEKK | 2.04 | 6.87E-02 | | 1.00E+00 | | 1.00E+00 | -1.20 | 7.36E-02 |
| AT5G56030 | (a) | EIS(ph)DDEEEEEKKDEEGK | 0.73 | 1.43E-01 | 1.11 | 2.55E-01 | -0.41 | 5.70E-01 | -0.04 | 3.22E-01 |
| AT5G56030 | (a) | TIEKEIS(ph)DDEEEEEK | | 1.00E+00 | 0.49 | 6.10E-01 | -0.32 | 5.56E-01 | | 1.00E+00 |

## transport.

(ABC) ABC transorters; (aa) amino acids; (am) ammonium; (Ca) calcium; (H+) H+ transportig pyrophosphatase; (Aq) Major Intrinsic Proteins; (U) membrane system unknown; (met) metabolite transporters; (m) metall; (mi) misc; (ni) nitrate; (nu) nucleotides; (ATP) p- and v-ATPases; (pep) peptides; (px) peroxisomes; (P) phosphate; (K) potassium; (su) sugars; (S) sulphate; (-) anions; (+) cations

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G02520 | (ABC) | TSELSSGS(ph)SFR | -0.06 | 9.50E-01 | 0.39 | 2.36E-01 | -0.93 | 1.10E-01 | -0.49 | 1.81E-01 |
| AT1G08090 | (ni) | EQSFAFSVQS(ph)PIVHTDK | | 4.22E-02 | | 1.00E+00 | | 1.00E+00 | | 4.89E-01 |
| AT1G11670 | (mi) | (ac)GSEATTAVNNLQQPLLESTKS(ph)EADFR | -1.72 | 8.97E-02 | | 1.00E+00 | | 1.00E+00 | 1.36 | 1.00E+00 |
| AT1G15210 | (ABC) | NM(ox)EDIFNT(ph)SSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G15210 | (ABC) | NM(ox)EDIFNTSS(ph)R | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G15210 | (ABC) | NMEDIFNT(ph)SSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G15500 | (met) | AS(ph)SVKIPVVSQEDAPSGETTSQLSEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 2.04E-01 |
| AT1G15690 | (H+) | LTSDLGASS(ph)SGGANNGK | 0.39 | 7.04E-01 | -0.41 | 1.00E+00 | 0.54 | 2.43E-01 | -0.26 | 3.01E-02 |
| AT1G16010 | (+) | SNDGLSVSAPVS(ph)PVSS(ph)PPDSR | 0.91 | 1.23E-01 | 0.35 | 1.28E-01 | -0.74 | 1.15E-01 | -1.29 | 8.63E-02 |
| AT1G17840 | (ABC) | NGTQNTTVAPDGLTQSPS(ph)LR | 0.46 | 1.11E-01 | 0.76 | 2.29E-02 | -0.36 | 1.98E-01 | -0.07 | 7.84E-01 |
| AT1G18880 | (pep) | TSAEFDKVS(ph)V | | 1.00E+00 | | 1.00E+00 | -0.79 | 1.67E-01 | | 1.00E+00 |
| AT1G19450 | (su) | (ac)S(ph)FRDDNTEEGR | 0.76 | 2.30E-01 | 0.58 | 2.77E-01 | -0.35 | 5.77E-01 | -0.52 | 4.88E-01 |
| AT1G19450 | (su) | (ac)S(ph)FRDDNTEEGRNDLR | 0.46 | 5.23E-01 | 0.79 | 1.52E-01 | -0.33 | 3.61E-01 | 0.00 | 9.80E-01 |
| AT1G19450 | (su) | QSSM(ox)LES(ph)SQVIR | 0.52 | 2.80E-01 | 0.85 | 1.94E-01 | -0.24 | 5.84E-01 | 0.09 | 9.96E-02 |
| AT1G20840 | (su) | DNDDYATDDGAGDDDDS(ph)DNDLRSPLM(ox)SR | 0.23 | 5.34E-01 | 1.22 | 1.54E-02 | -0.97 | 4.16E-02 | 0.02 | 9.05E-01 |
| AT1G20840 | (su) | LYGTHENQSYLARPVPEQNS(ph)SLGLR | | 1.00E+00 | | 1.00E+00 | | 3.52E-02 | | 1.00E+00 |
| AT1G20840 | (su) | PVPEQNS(ph)SLGLR | 0.59 | 2.22E-01 | 1.70 | 3.11E-02 | -0.82 | 1.66E-01 | 0.29 | 2.03E-01 |
| AT1G20840 | (su) | YYLKEDGAES(ph)R | | 1.00E+00 | 0.73 | 1.56E-01 | -0.93 | 1.25E-01 | | 1.00E+00 |
| AT1G22530 | (mi) | EILQSES(ph)FKEEGYLASELQEAEK | -3.00 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.59 | 1.00E+00 |
| AT1G22530 | (mi) | TFGSIIT(ph)SPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G22530 | (mi) | TFGSIITS(ph)PR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G25530 | (aa) | VSSS(ph)PVS(ph)PSKETDRK | 0.34 | 6.80E-01 | 0.03 | 1.00E+00 | -0.32 | 6.47E-01 | -0.63 | 4.19E-01 |
| AT1G30450 | (-) | SMTGEQIQAPS(ph)SPR | 0.68 | 6.80E-02 | -0.25 | 3.95E-01 | 0.44 | 1.76E-01 | -0.49 | 1.47E-01 |
| AT1G30560 | (U) | LGDTITES(ph)FLSSR | -0.55 | 1.16E-01 | 1.97 | 2.18E-02 | -0.06 | 8.30E-01 | 2.47 | 7.26E-03 |
| AT1G31830 | (aa) | (ac)SQYENNEVPYSSVGADEVPS(ph)SPPKATDK | 0.09 | 9.31E-01 | 0.62 | 9.39E-02 | -0.01 | 4.48E-01 | 0.52 | 2.37E-01 |
| AT1G31830 | (aa) | (ac)SQYENNEVPYSSVGADEVPSS(ph)PPK | 0.14 | 6.44E-01 | 0.91 | 6.52E-02 | -0.25 | 4.99E-01 | 0.51 | 2.54E-01 |
| AT1G48370 | (pep) | QIEEHELQETGIS(ph)PDIER | 0.36 | 4.28E-01 | -0.13 | 3.88E-01 | 0.64 | 1.33E-01 | 0.15 | 7.80E-01 |
| AT1G53390 | (ABC) | TQS(ph)QIFK | | 1.00E+00 | 0.18 | 1.00E+00 | -0.13 | 3.45E-01 | | 1.00E+00 |
| AT1G57980 | (nu) | M(ox)VEFNQS(ph)ENNVEV | 0.27 | 1.90E-02 | 1.00 | 1.82E-02 | -0.96 | 1.63E-02 | -0.23 | 2.68E-01 |
| AT1G59870 | (ABC) | (ac)M(ox)DYNPNLPPLGGGGVS(ph)M(ox)RR | -0.78 | 3.15E-02 | 0.99 | 1.02E-02 | -1.20 | 3.91E-03 | 0.56 | 8.87E-02 |
| AT1G59870 | (ABC) | LPT(ph)YSR | 0.66 | 2.58E-01 | 0.61 | 3.53E-01 | -0.08 | 9.87E-01 | -0.13 | 7.89E-01 |
| AT1G59870 | (ABC) | NIEDIFSS(ph)GSRR | 0.39 | 6.37E-01 | -0.61 | 5.41E-01 | 0.23 | 9.23E-01 | -0.77 | 3.07E-01 |
| AT1G59870 | (ABC) | NIEDIFSSGS(ph)R | 0.52 | 1.59E-01 | 0.68 | 4.34E-02 | -0.50 | 9.37E-02 | -0.34 | 3.04E-01 |
| AT1G59870 | (ABC) | SLS(ph)TADGNR | -0.30 | 3.71E-01 | 1.15 | 5.78E-01 | -0.90 | 6.84E-01 | 0.55 | 4.52E-01 |
| AT1G59870 | (ABC) | SLS(ph)TADGNRR | 0.19 | 3.75E-01 | -0.45 | 7.04E-02 | -0.24 | 2.11E-01 | -0.88 | 1.34E-03 |
| AT1G59870 | (ABC) | TQS(ph)VNDDEEALK | 0.81 | 7.58E-02 | 0.65 | 7.38E-02 | -0.07 | 3.37E-01 | -0.23 | 5.30E-01 |
| AT1G59870 | (ABC) | TQS(ph)VNDDEEALKWAAIEK | -1.65 | 3.19E-01 | | 1.00E+00 | | 1.00E+00 | 1.30 | 6.72E-01 |
| AT1G64780 | (am) | ISAEDEMAGMDMT(ph)R | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G69870 | (pep) | ISS(ph)PGSILDAEK | 1.05 | 1.61E-01 | 1.62 | 3.15E-02 | -0.23 | 7.64E-01 | 0.34 | 5.99E-01 |
| AT1G69870 | (pep) | ISS(ph)PGSILDAEKVEK | -0.45 | 1.57E-01 | 0.51 | 4.12E-01 | 1.05 | 2.67E-01 | 2.02 | 1.09E-01 |
| AT1G69870 | (pep) | SGS(ph)FSKS(ph)SPSELDVVDPYKR | 0.14 | 7.17E-01 | -0.17 | 7.03E-01 | 0.82 | 1.87E-01 | 0.51 | 5.05E-01 |
| AT1G69870 | (pep) | VGLPIEDFEEDKS(ph)SDDVEM(ox)TSK | -0.38 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G71090 | (mi) | VFNSISSFSQTSFPEVDLGGEYGGESSS(ph)PR | | 1.00E+00 | | 4.71E-01 | | 1.00E+00 | 0.05 | 1.00E+00 |
| AT1G71880 | (su) | DAAALETQS(ph)PEDFDQPSPLR | 0.11 | 4.40E-01 | 0.40 | 4.50E-02 | 0.32 | 1.48E-01 | 0.61 | 9.77E-03 |
| AT1G71880 | (su) | DAAALETQS(ph)PEDFDQPSPLRK | 0.03 | 8.10E-01 | -0.18 | 6.71E-01 | 0.30 | 4.59E-01 | 0.09 | 9.32E-01 |
| AT1G75220 | (su) | (ac)S(ph)FRDDNEEAR | 0.10 | 8.39E-01 | -0.98 | 8.98E-02 | 1.04 | 6.86E-02 | -0.05 | 9.77E-01 |
| AT1G75220 | (su) | (ac)S(ph)FRDDNEEARNDLR | | 1.00E+00 | -0.90 | 6.67E-02 | 0.60 | 1.19E-01 | | 1.00E+00 |
| AT1G75220 | (su) | QSSM(ox)M(ox)GS(ph)SQVIR | | 1.00E+00 | -0.51 | 1.20E-01 | | 1.00E+00 | -0.47 | 1.37E-01 |
| AT1G80830 | (m) | S(ph)FSNSPLIENSDSNQIIVSEK | -1.01 | 1.53E-01 | | 1.00E+00 | 1.32 | 1.44E-01 | | 1.00E+00 |
| AT2G01980 | (+) | SVS(ph)FGGIYNNK | 0.40 | 4.88E-01 | 1.05 | 7.31E-02 | -0.63 | 2.97E-01 | 0.02 | 7.82E-01 |
| AT2G03240 | (P) | AFS(ph)GLIS(ph)TSPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G04100 | (mi) | TSS(ph)FGNGLA | 0.39 | 2.62E-01 | 1.07 | 9.49E-03 | -0.26 | 5.38E-01 | 0.42 | 2.14E-01 |
| AT2G16850 | (Aq) | ALAS(ph)FRS(ph)NPTN | -0.22 | 6.00E-01 | 0.72 | 2.24E-02 | -1.04 | 3.86E-03 | -0.10 | 5.49E-01 |
| AT2G18960 | (ATP) | (ac)S(ph)GLEDIKNETVDLEK | -0.48 | 1.28E-01 | | 1.00E+00 | 1.92 | 1.77E-02 | | 1.00E+00 |
| AT2G18960 | (ATP) | EDVNIFPEKGS(ph)YR | 0.20 | 7.09E-01 | 0.83 | 2.35E-01 | -0.42 | 6.14E-01 | 0.20 | 7.20E-01 |
| AT2G18960 | (ATP) | GLDIDTAGHHYT(ph)V | 0.08 | 2.64E-01 | -0.64 | 7.07E-01 | 2.04 | 2.35E-01 | 1.32 | 9.53E-01 |
| AT2G18960 | (ATP) | T(ph)LHGLQPK | 0.35 | 4.81E-01 | 0.10 | 6.57E-01 | 1.09 | 1.54E-02 | 0.84 | 5.66E-02 |
| AT2G25520 | (met) | VQAS(ph)DDEAGKLLEER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G26910 | (ABC) | STS(ph)FKDEIEDEEELR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.55 | 3.93E-01 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT2G28910 | (Ca) | SLS(ph)ES(ph)EDEEEGR | 0.15 | 8.15E-01 | 0.41 | 5.88E-01 | -0.50 | 5.77E-01 | -0.24 | 8.04E-01 |
| AT2G30070 | (K) | LSTYATGS(ph)PGETR | 0.02 | 4.09E-01 | | 1.00E+00 | | 1.00E+00 | -0.67 | 9.62E-02 |
| AT2G36380 | (ABC) | S(ph)FRDVFAPPTDDVFGR | -1.44 | 2.45E-01 | | 1.00E+00 | | 1.00E+00 | 0.71 | 6.32E-01 |
| AT2G36910 | (ABC) | NSVSS(ph)PIM(ox)TR | 0.15 | 6.19E-01 | 0.26 | 3.35E-01 | -0.63 | 5.75E-02 | -0.51 | 1.58E-01 |
| AT2G38940 | (P) | SLEEM(ox)SGENEDNENS(ph)NNDSR | 0.90 | 3.62E-02 | 0.69 | 8.14E-02 | -0.07 | 5.79E-01 | -0.28 | 3.36E-01 |
| AT2G39010 | (Aq) | S(ph)QLHELHA | -0.18 | 6.93E-01 | 1.12 | 4.99E-02 | -0.60 | 2.29E-01 | 0.70 | 1.49E-01 |
| AT2G39010 | (Aq) | TKDELTEEES(ph)LSGK | -1.73 | 1.00E+00 | 0.93 | 1.10E-01 | 0.17 | 1.00E+00 | 1.03 | 1.00E+00 |
| AT2G39130 | (aa) | FGSSFLS(ph)SGLIR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G39130 | (aa) | LSS(ph)QGLLSPIPSR | 0.31 | 2.98E-01 | 0.91 | 9.76E-03 | -0.53 | 8.40E-02 | 0.08 | 8.31E-01 |
| AT2G39130 | (aa) | LSSQGLLS(ph)PIPSR | -0.03 | 8.97E-01 | 0.69 | 1.92E-02 | -0.65 | 1.41E-02 | 0.07 | 8.26E-01 |
| AT2G39480 | (ABC) | GFQEPSS(ph)PK | 1.03 | 4.61E-03 | 0.00 | 4.55E-01 | 0.04 | 9.39E-03 | -0.99 | 6.11E-03 |
| AT2G39480 | (ABC) | RQDS(ph)FEM(ox)R | 1.01 | 8.37E-03 | 0.10 | 9.67E-01 | -0.47 | 5.45E-01 | -1.38 | 2.92E-02 |
| AT2G39480 | (ABC) | SNGSDPES(ph)PISPLLISDPQNER | 0.74 | 7.52E-02 | -0.08 | 6.48E-01 | 0.40 | 2.23E-01 | -0.42 | 2.16E-01 |
| AT2G39970 | (px) | DQTAAPES(ph)PSSNAEALVAVEPR | 0.22 | 3.88E-01 | 0.86 | 1.33E-02 | -0.31 | 3.30E-01 | 0.34 | 2.58E-01 |
| AT2G39970 | (px) | DQTAAPES(ph)PSSNAEALVAVEPRPYGTFNTIR | -1.21 | 4.02E-02 | | 1.00E+00 | | 1.00E+00 | 0.36 | 2.51E-01 |
| AT2G47000 | (ABC) | (ac)AS(ph)ESGLNGDPNILEEVSETKR | -0.19 | 8.15E-01 | -0.15 | 3.98E-01 | 0.69 | 1.50E-01 | 0.73 | 3.62E-01 |
| AT2G47000 | (ABC) | GNS(ph)S(ph)RHS(ph)FNM(ox)FGFPAGIDGNVVQDQEEDDTTQPK | -1.16 | 1.52E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G47000 | (ABC) | M(ox)SSIES(ph)FKQS(ph)SLRK | -0.70 | 2.70E-01 | | 1.00E+00 | | 1.00E+00 | 0.38 | 3.83E-01 |
| AT2G47000 | (ABC) | MSSIES(ph)FKQS(ph)SLR | 1.41 | 2.23E-02 | 0.77 | 1.89E-01 | -0.05 | 8.75E-01 | -0.68 | 1.59E-01 |
| AT2G47160 | (-) | SLSQVFS(ph)PR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G47800 | (ABC) | GITGLVTAET(ph)NSPTKPSDAVSVEK | | 1.00E+00 | 0.29 | 8.41E-01 | | 1.00E+00 | 0.47 | 7.56E-01 |
| AT2G47800 | (ABC) | TPT(ph)SPHASSPR | 2.82 | 4.90E-04 | 0.71 | 6.42E-02 | 0.16 | 6.37E-01 | -1.96 | 1.13E-02 |
| AT3G06450 | (-) | VVS(ph)FQNPR | -0.03 | 4.35E-01 | 0.38 | 6.28E-01 | -0.48 | 6.18E-01 | -0.07 | 4.75E-01 |
| AT3G12100 | (m) | SIS(ph)FNPR | 0.49 | 3.00E-02 | -0.37 | 1.93E-01 | -0.30 | 4.92E-02 | -1.16 | 3.11E-02 |
| AT3G17650 | (pep) | TSFTLEEDPHAS(ph)PLS(ph)PK | -0.94 | 2.48E-01 | 0.15 | 9.47E-01 | -0.09 | 8.40E-01 | 1.01 | 2.94E-01 |
| AT3G21250 | (ABC) | GNS(ph)SQNLQL | -0.01 | 9.47E-01 | 0.10 | 1.56E-01 | -0.28 | 2.05E-01 | -0.17 | 4.90E-01 |
| AT3G23870 | (nu) | DTPVFTNS(ph)GSGR | 1.32 | 1.01E-01 | 0.88 | 4.23E-02 | 0.32 | 3.23E-01 | -0.12 | 4.32E-01 |
| AT3G24300 | (am) | ISEQHEM(ox)QGM(ox)DM(ox)T(ph)R | | 1.00E+00 | -0.29 | 2.98E-01 | 1.06 | 2.45E-02 | | 1.00E+00 |
| AT3G24300 | (am) | SAT(ph)PPRV | 1.10 | 4.52E-02 | 0.79 | 1.14E-01 | -0.53 | 2.71E-01 | -0.83 | 1.01E-01 |
| AT3G24300 | (am) | VDPGS(ph)PFPR | 0.52 | 1.69E-01 | 1.11 | 4.09E-01 | 0.11 | 7.19E-01 | 0.70 | 2.56E-01 |
| AT3G25610 | (ATP) | S(ph)GGSPLVNEDLDVVVDQSGPK | -0.61 | 9.61E-01 | -1.87 | 7.19E-02 | 1.44 | 2.08E-01 | 0.19 | 7.96E-01 |
| AT3G28345 | (ABC) | SSS(ph)ANSVTGPSTIK | 0.04 | 4.25E-01 | | 1.00E+00 | | 1.00E+00 | -0.95 | 7.17E-02 |
| AT3G28860 | (ABC) | DFSNPS(ph)TRR | 1.11 | 1.09E-02 | -0.51 | 2.25E-01 | 0.21 | 1.84E-01 | -1.41 | 4.61E-02 |
| AT3G28860 | (ABC) | DFSNPST(ph)R | -0.30 | 5.58E-02 | -0.90 | 1.00E+00 | 0.50 | 3.39E-02 | -0.10 | 1.00E+00 |
| AT3G28860 | (ABC) | NLSYSYS(ph)TGADGR | | 5.58E-02 | | 1.00E+00 | | 3.39E-02 | | 1.00E+00 |
| AT3G47420 | (U) | DEVLDSS(ph)SEK | 0.65 | 1.91E-01 | 1.57 | 1.00E+00 | -1.10 | 5.43E-02 | -0.18 | 1.00E+00 |
| AT3G47420 | (U) | IGNSVNEPLLLSSS(ph)DSETDDKKR | 0.12 | 9.81E-01 | 0.25 | 4.90E-01 | 0.75 | 6.78E-01 | 0.88 | 2.09E-01 |
| AT3G53420 | (Aq) | SLGS(ph)FRS(ph)AANV | 0.72 | 8.82E-02 | 0.72 | 3.44E-01 | -0.94 | 1.94E-01 | -0.94 | 4.56E-02 |
| AT3G53480 | (ABC) | S(ph)TLLDDGDESM(ox)TEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G53720 | (m) | DLSASQDS(ph)TKEELR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G54820 | (Aq) | ALGS(ph)FRS(ph)QPHV | 1.64 | 1.19E-01 | -0.22 | 4.91E-01 | -0.28 | 8.57E-01 | -2.14 | 5.87E-02 |
| AT3G55320 | (ABC) | SNGSEPES(ph)PVS(ph)PLLTSDPK | -0.70 | 7.70E-02 | 0.78 | 1.76E-02 | -1.72 | 4.32E-04 | -0.24 | 2.14E-01 |
| AT3G58730 | (ATP) | GIS(ph)INAAR | -0.02 | 9.40E-01 | 1.19 | 4.73E-02 | -1.03 | 6.00E-02 | 0.18 | 8.07E-01 |
| AT3G59140 | (ABC) | FDESGESSLYEPLNAGDS(ph)NGFSEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G62150 | (ABC) | (ac)M(ox)DSVIESEEGLKVDS(ph)PNR | 0.26 | 5.25E-01 | 1.08 | 3.55E-03 | -0.80 | 2.07E-02 | 0.03 | 8.24E-01 |
| AT3G62150 | (ABC) | (ac)M(ox)DSVIESEEGLKVDS(ph)PNRADAETSNSK | -0.41 | 2.34E-01 | 0.43 | 2.25E-01 | -0.38 | 2.91E-01 | 0.47 | 1.83E-01 |
| AT3G62150 | (ABC) | S(ph)SSFSM(ox)FGFPAGIDTNNEAIPEKDIK | -0.70 | 3.74E-01 | | 1.00E+00 | | 1.00E+00 | -0.81 | 2.27E-01 |
| AT3G62150 | (ABC) | SSSFS(ph)M(ox)FGFPAGIDTNNEAIPEK | -0.84 | 8.11E-02 | | 1.00E+00 | | 1.00E+00 | 1.33 | 3.63E-02 |
| AT3G62270 | (-) | NLS(ph)QVFS(ph)PR | -0.12 | 2.86E-03 | 0.41 | 5.93E-02 | -0.36 | 1.73E-01 | 0.17 | 1.00E+00 |
| AT3G62700 | (ABC) | SIS(ph)IES(ph)PR | 0.41 | 3.65E-01 | 1.62 | 4.93E-03 | -1.65 | 3.90E-03 | -0.43 | 2.76E-01 |
| AT3G62700 | (ABC) | SIS(ph)IES(ph)PRQPK | -0.14 | 5.31E-01 | 0.78 | 5.21E-03 | -0.87 | 2.88E-03 | 0.05 | 9.40E-01 |
| AT3G62700 | (ABC) | SIS(ph)IES(ph)PRQPKS(ph)PK | | 1.00E+00 | 0.06 | 3.94E-01 | | 1.00E+00 | 0.03 | 4.27E-01 |
| AT3G62700 | (ABC) | SIS(ph)IESPRQPK | -1.05 | 5.29E-01 | 0.11 | 9.64E-01 | -1.31 | 3.43E-01 | -0.16 | 6.23E-01 |
| AT3G62700 | (ABC) | TTS(ph)MESPR | -0.68 | 2.74E-01 | 1.44 | 1.00E+00 | -0.70 | 1.00E+00 | 1.42 | 1.46E-01 |
| AT3G62770 | (mi) | IAPDATPSS(ph)PSSSLSLFK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G00630 | (K) | LQVALESLEAEGYNTS(ph)EESEVR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G13510 | (am) | HGGFAYM(ox)YFDDDES(ph)HK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G13510 | (am) | ISSEDEM(ox)AGM(ox)DM(ox)T(ph)R | 0.64 | 9.09E-02 | 0.93 | 1.34E-02 | -1.00 | 1.52E-02 | -0.71 | 1.01E-01 |
| AT4G13510 | (am) | ISSEDEMAGMDMT(ph)R | 1.62 | 2.53E-03 | 1.10 | 1.01E-02 | 0.54 | 1.12E-01 | 0.02 | 8.72E-01 |
| AT4G13510 | (am) | RVEPRS(ph)PS(ph)PSGANTTPTPV | | 1.00E+00 | 0.82 | 1.18E-01 | -0.76 | 1.56E-01 | | 1.00E+00 |
| AT4G13510 | (am) | S(ph)PS(ph)PSGANTTPTPV | 0.62 | 4.01E-01 | 1.62 | 4.22E-02 | -0.89 | 2.58E-01 | 0.10 | 8.30E-01 |
| AT4G18910 | (Aq) | SGS(ph)FLK | | 1.00E+00 | 1.02 | 6.79E-02 | -0.68 | 1.25E-01 | | 1.00E+00 |
| AT4G23640 | (K) | SIS(ph)EANIAGSSR | -0.15 | 3.37E-01 | 0.11 | 3.37E-01 | -0.40 | 1.40E-01 | -0.13 | 3.23E-01 |
| AT4G23700 | (m) | NVTTEESLVEDSES(ph)P | 0.95 | 7.77E-02 | 1.14 | 1.13E-01 | -0.76 | 2.98E-01 | -0.57 | 2.13E-01 |
| AT4G30190 | (ATP) | EAVNIFPEKGS(ph)YR | -0.30 | 5.62E-01 | 0.22 | 5.74E-01 | -0.09 | 7.11E-01 | 0.43 | 4.30E-01 |
| AT4G30190 | (ATP) | GLDIETPSHYT(ph)V | 0.74 | 1.40E-01 | 0.48 | 4.88E-01 | 0.95 | 3.57E-02 | 0.69 | 2.50E-01 |
| AT4G33530 | (K) | TSPAVDS(ph)FDVDALEIPGTQK | 0.71 | 1.21E-02 | -0.15 | 9.72E-02 | 0.75 | 2.33E-02 | -0.11 | 5.98E-01 |
| AT4G35100 | (Aq) | ALGS(ph)FRSNATN | -0.34 | 1.69E-01 | -0.14 | 8.41E-01 | -0.80 | 1.03E-01 | -0.60 | 9.32E-02 |
| AT4G35300 | (su) | GGS(ph)TM(ox)SVLSR | | 1.00E+00 | 0.09 | 3.38E-01 | 0.05 | 4.98E-01 | | 1.00E+00 |
| AT4G38640 | (mi) | FAAIYDSSS(ph)PSHPLLSKPSTSALDS(ph)PR | | 2.09E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G38640 | (mi) | PSTSALDS(ph)PR | 1.14 | 6.92E-02 | 0.82 | 2.70E-01 | -0.46 | 6.22E-01 | -0.78 | 5.05E-02 |
| AT4G39080 | (ATP) | ET(ph)ESQQAGEDLLESPLLQEEK | 0.66 | 1.66E-01 | -0.80 | 1.83E-01 | 1.52 | 3.51E-02 | 0.06 | 6.07E-01 |
| AT4G39080 | (ATP) | ETESQQAGEDLLES(ph)PLLQEEK | 0.17 | 6.58E-01 | -0.34 | 2.63E-01 | 0.86 | 4.88E-02 | 0.35 | 5.28E-01 |
| AT5G01490 | (Ca) | TVS(ph)ASSLIR | 2.05 | 7.10E-03 | 0.94 | 9.47E-02 | 0.12 | 7.91E-01 | -0.99 | 1.00E+00 |
| AT5G02170 | (aa) | QSSVFGSFT(ph)SSPSK | | 1.00E+00 | | 1.00E+00 | 0.16 | 8.52E-02 | | 1.00E+00 |
| AT5G03280 | (m) | AAPTSNFTVGSDGPPS(ph)FR | 0.03 | 6.75E-01 | 0.93 | 6.19E-03 | -0.90 | 8.14E-03 | 0.01 | 7.78E-01 |
| AT5G03280 | (m) | KYS(ph)SM(ox)PDISGLSM(ox)SAR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G03280 | (m) | SLSGEGGS(ph)GTGS(ph)LSR | -0.51 | 8.33E-02 | 0.67 | 1.76E-01 | -0.85 | 1.01E-01 | 0.32 | 1.56E-01 |
| AT5G03280 | (m) | SLSGEGGS(ph)GTGSLSR | -0.24 | 5.21E-01 | -0.06 | 4.96E-01 | -0.47 | 7.48E-02 | -0.29 | 5.26E-01 |
| AT5G03280 | (m) | SLSGEGGSGTGS(ph)LSR | 0.28 | 2.82E-01 | -0.24 | 3.07E-01 | 0.15 | 1.39E-01 | -0.37 | 2.39E-01 |
| AT5G04930 | (ATP) | EVTFGDLGS(ph)K | -0.16 | 9.05E-01 | 1.17 | 9.39E-02 | -0.86 | 1.98E-01 | 0.47 | 4.82E-01 |
| AT5G04930 | (ATP) | EVTFGDLGS(ph)KR | -0.14 | 3.33E-01 | 0.61 | 5.28E-01 | -1.36 | 6.23E-02 | -0.61 | 3.53E-02 |
| AT5G09400 | (K) | SLESDGNDDS(ph)DS(ph)EEDFPGSR | 0.29 | 7.72E-01 | 0.58 | 3.55E-01 | -0.80 | 9.50E-02 | -0.51 | 3.17E-01 |
| AT5G09400 | (K) | VDS(ph)FDVEALEVPGAPR | -0.13 | 8.97E-01 | 0.26 | 2.81E-01 | 0.26 | 1.28E-01 | 0.66 | 2.39E-01 |
| AT5G13550 | (S) | (ac)SYASLS(ph)VK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G13550 | (-) | YGGSNNNSS(ph)SSNALLKEPLLSVEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G14880 | (K) | SDKPLNYS(ph)PDPENESGINER | 0.63 | 2.64E-01 | 0.07 | 7.78E-01 | 0.20 | 8.21E-01 | -0.36 | 5.09E-01 |
| AT5G17010 | (su) | SSGEIS(ph)PEREPLIK | 1.38 | 3.75E-04 | 1.25 | 6.71E-04 | -0.37 | 2.05E-01 | -0.50 | 7.77E-02 |
| AT5G20650 | (m) | SSS(ph)GVSAPLIPK | 0.48 | 2.39E-01 | 1.25 | 2.03E-01 | -0.15 | 8.91E-01 | 0.62 | 9.80E-01 |
| AT5G24030 | (met) | NVS(ph)SENIENYLK | -0.26 | 4.81E-01 | 0.01 | 4.68E-01 | -0.05 | 1.00E+00 | 0.21 | 9.94E-01 |
| AT5G24380 | (m) | TNS(ph)ISTLVEVPEDSTK | | 1.00E+00 | | 1.00E+00 | -0.41 | 1.27E-01 | | 1.00E+00 |
| AT5G27150 | (+) | GFVPFVPGS(ph)PTER | 0.35 | 1.89E-02 | 0.74 | 1.32E-04 | 0.17 | 3.28E-01 | 0.56 | 4.85E-03 |
| AT5G27150 | (+) | GFVPFVPGS(ph)PTERNPPDLSK | -1.12 | 2.90E-01 | -1.15 | 9.60E-02 | 0.93 | 3.02E-01 | 0.90 | 6.15E-01 |
| AT5G27150 | (+) | GFVPFVPGS(ph)PTERNPPDLSKA | -1.46 | 8.35E-02 | -2.32 | 3.23E-03 | 1.76 | 1.08E-02 | 0.90 | 3.38E-01 |
| AT5G40890 | (-) | TLS(ph)STPLALVGAK | 0.01 | 6.05E-01 | 1.16 | 3.08E-02 | -0.20 | 6.54E-01 | 0.94 | 1.39E-01 |
| AT5G43370 | (P) | SLEELSGEAEVS(ph)HDEK | 0.19 | 7.16E-01 | 0.67 | 3.90E-01 | -0.31 | 9.30E-01 | 0.16 | 6.71E-01 |
| AT5G44240 | (ATP) | DLS(ph)PISITQPK | 1.51 | 1.78E-02 | 1.79 | 4.84E-03 | -0.48 | 3.24E-01 | -0.21 | 6.98E-01 |
| AT5G44240 | (ATP) | SPVYEPLLS(ph)DSPNATRR | | 1.00E+00 | | 1.00E+00 | 0.33 | 2.15E-01 | | 1.00E+00 |
| AT5G44240 | (ATP) | SPVYEPLLSDS(ph)PNATRR | -0.99 | 3.38E-01 | | 1.00E+00 | | 1.00E+00 | 0.80 | 7.39E-01 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT5G45380 | (+) | VVEAYAS(ph)GDEDVDVPAEELREEK | -0.83 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.55 | 1.00E+00 |
| AT5G49890 | (-) | KIS(ph)GILDDGSVGFR | -1.69 | 2.14E-01 | -0.93 | 2.29E-03 | 0.86 | 1.14E-01 | 1.62 | 3.89E-01 |
| AT5G49890 | (-) | TTFGS(ph)QILR | 1.57 | 4.36E-03 | 1.46 | 1.34E-02 | -0.47 | 3.89E-01 | -0.58 | 1.31E-01 |
| AT5G57110 | (Ca) | (ac)TSLLKS(ph)SPGRR | | 1.00E+00 | -1.30 | 1.00E+00 | | 8.00E-02 | | 1.00E+00 |
| AT5G57110 | (Ca) | (ac)TSLLKSS(ph)PGR | -0.72 | 1.61E-01 | -2.45 | 2.36E-03 | 1.61 | 1.65E-01 | -0.12 | 3.10E-01 |
| AT5G57110 | (Ca) | GGDVESGKSEHADS(ph)DSDTFYIPSK | | 1.61E-01 | | 2.36E-03 | | 1.65E-01 | | 3.10E-01 |
| AT5G57110 | (Ca) | S(ph)EHADSDSDTFYIPSK | | 1.00E+00 | -0.64 | 4.12E-02 | 1.16 | 5.49E-02 | | 1.00E+00 |
| AT5G59520 | (m) | SGVDVS(ph)QALIR | | 1.00E+00 | -0.11 | 6.32E-01 | 0.47 | 1.44E-01 | | 1.00E+00 |
| AT5G60660 | (Aq) | ALGS(ph)FGS(ph)FGSFR | -0.38 | 1.62E-01 | 0.57 | 1.20E-01 | -0.29 | 2.43E-01 | 0.66 | 7.74E-02 |
| AT5G60660 | (Aq) | ALGSFGS(ph)FGS(ph)FR | -0.13 | 4.69E-01 | 0.70 | 9.30E-01 | 0.03 | 7.05E-01 | 0.86 | 6.59E-01 |
| AT5G61520 | (su) | DIQETTILS(ph)H | | 1.00E+00 | 0.65 | 2.31E-01 | 0.12 | 4.95E-01 | | 1.00E+00 |
| AT5G62670 | (ATP) | GLDIETIQQAYT(ph)V | 0.24 | 1.81E-01 | 0.23 | 2.32E-01 | 1.17 | 1.18E-04 | 1.16 | 1.41E-04 |
| AT5G62670 | (ATP) | LKGLDIETIQQAYT(ph)V | -0.88 | 5.05E-01 | | 1.00E+00 | | 1.00E+00 | 0.48 | 9.28E-01 |
| AT5G64410 | (pep) | (ac)ATADEFS(ph)DEDTSPIEEVR | 0.40 | 4.77E-02 | 1.14 | 4.85E-06 | -0.31 | 9.30E-02 | 0.44 | 3.17E-02 |

## others - amino acid metabolism

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G17745 | FSTVGS(ph)DSDEYNPTLPKPR | -0.43 | 4.50E-01 | 0.33 | 4.96E-01 | 0.22 | 7.40E-01 | 0.97 | 1.01E-01 |
| AT1G17745 | TVEQTTLTEDNRFS(ph)TVGSDSDEYNPTLPKPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G39800 | QLVNSS(ph)FADLQKPQTELDGK | -0.83 | 6.76E-01 | -1.45 | 8.40E-02 | 1.79 | 5.24E-02 | 1.17 | 4.54E-01 |
| AT3G55610 | QLVNSS(ph)FADLQKPQM(ox)ELDGK | -1.27 | 1.94E-01 | -1.71 | 2.30E-02 | 1.34 | 4.69E-02 | 0.90 | 3.65E-01 |
| AT4G10760 | ASYPEIDVQPPS(ph)PPR | -0.28 | 6.43E-01 | 0.08 | 9.98E-01 | 0.68 | 4.71E-01 | 1.04 | 7.73E-01 |

## others - cell wall.

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G53840 | (ac)M(ox)DSVNS(ph)FKGYGK | 0.07 | 4.14E-01 | 0.45 | 2.20E-01 | -0.66 | 1.01E-01 | -0.28 | 2.26E-01 |
| AT2G46630 | APPT(ph)SPPQER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G46630 | SSYTS(ph)PPS(ph)PK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G03050 | M(ox)GGDS(ph)DDDEEM(ox)NLSLVPK | -0.09 | 9.09E-01 | 0.54 | 8.18E-02 | -0.54 | 1.00E-01 | 0.09 | 8.33E-01 |
| AT3G07330 | SSS(ph)DSGLTELSK | 0.26 | 6.94E-01 | | 1.00E+00 | | 1.00E+00 | -0.41 | 5.05E-01 |
| AT3G28180 | NS(ph)ESGLELLSK | 0.12 | 7.83E-01 | -0.07 | 7.53E-01 | -0.19 | 8.36E-01 | -0.38 | 4.36E-01 |
| AT3G28180 | RNS(ph)ESGLELLSK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G28180 | S(ph)SESDLLAFAEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G28180 | SS(ph)ESDLLAFAEKEEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G48530 | VEDLWDEQKPQLS(ph)PNEK | -0.69 | 5.50E-01 | 0.42 | 1.00E+00 | 0.67 | 1.28E-01 | 1.77 | 7.37E-02 |
| AT3G53520 | DEETIPM(ox)SQSSPYS(ph)PK | | 1.00E+00 | -1.22 | 1.97E-02 | | 9.67E-02 | -1.85 | 1.00E+00 |
| AT3G53520 | RDEETIPM(ox)SQSSPYS(ph)PK | | 1.00E+00 | 0.74 | 4.43E-01 | -0.51 | 3.36E-01 | | 1.00E+00 |
| AT4G28300 | SSVFPTSSYS(ph)PPEDSLSQDITDTVER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G32410 | NAISS(ph)PYIDPR | 1.06 | 9.79E-02 | 0.79 | 3.49E-01 | -0.40 | 6.08E-01 | -0.67 | 1.80E-01 |
| AT4G32410 | SDS(ph)NAPLFNM(ox)EDIDEGFEGYDDER | -1.53 | 5.43E-02 | -4.46 | 1.36E-02 | 2.93 | 1.00E+00 | 0.00 | 9.76E-01 |
| AT4G32410 | TTS(ph)GPLGPSDR | 0.91 | 1.43E-01 | 0.50 | 5.92E-01 | -0.50 | 6.21E-01 | -0.91 | 1.53E-01 |
| AT5G05170 | GGVDIDAS(ph)TDILADEALLNDEAR | 0.51 | 2.08E-01 | | 1.00E+00 | | 1.00E+00 | 0.77 | 2.49E-01 |
| AT5G05170 | LPYSSDVNQS(ph)PNR | 0.14 | 1.00E+00 | 0.35 | 3.25E-01 | -0.36 | 1.97E-01 | -0.15 | 1.65E-01 |
| AT5G05170 | LPYSSDVNQS(ph)PNRR | 0.57 | 2.59E-01 | 0.85 | 1.00E+00 | -1.52 | 1.00E+00 | -1.25 | 1.67E-01 |
| AT5G05170 | NTGPVSTQAAS(ph)ER | 0.68 | 2.28E-01 | 0.11 | 8.04E-01 | -0.34 | 5.39E-01 | -0.92 | 1.39E-01 |
| AT5G05170 | QDTSGEFSAAS(ph)PER | 1.08 | 5.05E-02 | 0.88 | 9.38E-02 | -0.32 | 5.27E-01 | -0.51 | 3.74E-01 |
| AT5G05170 | QDTSGEFSAAS(ph)PERLSVSSTIAGGK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |

## others - cell.

(c) cycle; (d) division; (u) unspecified

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| AGI | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G17130 | (c) | KSDGAANTSLASLFQNYGS(ph)DEDED | -0.56 | 3.43E-01 | -1.22 | 7.74E-02 | 0.88 | 1.80E-01 | 0.23 | 7.14E-01 |
| AT1G49040 | (d) | QQAISAGLPS(ph)PRPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G20000 | (d) | LHVPDEIDES(ph)P | 0.88 | 1.35E-01 | 0.29 | 1.00E+00 | 0.43 | 2.49E-01 | -0.17 | 1.00E+00 |
| AT2G20190 | (d) | ENS(ph)LFGGDADITEKPIEPIK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.47 | 4.16E-01 |
| AT2G20190 | (d) | SSS(ph)LDLGVDPPSSR | -0.15 | 1.00E+00 | 0.23 | 1.28E-01 | -0.75 | 1.00E+00 | -0.36 | 8.08E-02 |
| AT2G35110 | (d) | QYYPSQDESM(ox)S(ph)PTSVR | 0.50 | 4.80E-01 | | 1.00E+00 | | 1.00E+00 | -1.19 | 6.86E-03 |
| AT3G03790 | (d) | (ac)MELSVS(ph)PQTQK | | 1.00E+00 | 2.03 | 1.35E-01 | -0.35 | 1.00E+00 | | 1.00E+00 |
| AT3G10530 | (c) | VLPPVEQES(ph)DVELETK | 0.67 | 8.40E-02 | 0.03 | 8.22E-01 | 0.20 | 4.98E-01 | -0.43 | 1.81E-01 |
| AT3G12280 | (d) | VSVFPSVPDM(ox)S(ph)PK | 0.24 | 2.24E-01 | 0.20 | 6.51E-01 | -0.38 | 2.05E-01 | -0.42 | 4.03E-01 |
| AT3G63400 | (c) | NFS(ph)PGDVS(ph)DR | 0.37 | 4.62E-01 | 0.79 | 9.38E-02 | -0.27 | 5.41E-01 | 0.15 | 7.15E-01 |
| AT3G63400 | (c) | NFS(ph)PGDVS(ph)DREAK | 0.66 | 3.83E-03 | -0.16 | 3.95E-01 | 0.09 | 6.58E-01 | -0.74 | 1.57E-03 |
| AT4G19600 | (c) | IVGTADVTVS(ph)QSPK | 0.64 | 3.86E-02 | 0.71 | 5.25E-02 | -0.35 | 8.98E-02 | -0.28 | 1.29E-01 |
| AT4G28980 | (c) | LEDKDGETSEPPEVIPDYENS(ph)PR | -1.06 | 7.60E-02 | -0.25 | 1.00E+00 | 0.01 | 3.86E-01 | 0.81 | 1.00E+00 |
| AT4G32420 | (c) | RES(ph)PGS(ph)EEKGR | -2.16 | 6.41E-02 | 0.28 | 7.00E-01 | -1.69 | 1.46E-01 | 0.75 | 3.14E-01 |
| AT4G33060 | (c) | SFSVSDTVGNS(ph)DDDDDGEDETKFDAK | -0.53 | 7.53E-02 | | 1.00E+00 | | 1.00E+00 | 0.30 | 2.19E-01 |
| AT5G12350 | (d) | SDGTPSEANS(ph)PR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G13850 | (u) | IDLDKPEVEDDDDNDEDDS(ph)EDDDEAEGHDGEAGGR | 0.02 | 7.79E-01 | 0.61 | 2.24E-02 | 0.48 | 1.06E-01 | 1.07 | 2.87E-04 |
| AT5G13850 | (u) | LEEQKIDLDKPEVEDDDDNDEDDS(ph)EDDDEAEGHDGEAGGR | -2.07 | 4.31E-02 | -0.22 | 8.23E-01 | 0.42 | 6.45E-01 | 2.26 | 1.43E-02 |
| AT5G45190 | (c) | NVDVGDALISQS(ph)PK | 1.16 | 1.98E-03 | 0.21 | 6.75E-01 | -0.13 | 8.35E-01 | -1.07 | 3.11E-03 |
| AT5G61960 | (d) | VIHNSIGS(ph)PVNSFIER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G64960 | (c) | EIVTS(ph)PGR | 0.59 | 7.43E-02 | 0.60 | 3.04E-01 | -0.23 | 6.65E-01 | -0.21 | 3.21E-02 |
| AT5G64960 | (c) | SYSHDHTGNLT(ph)NR | | 7.43E-02 | | 1.00E+00 | | 1.00E+00 | | 3.21E-02 |

## others - DNA.

(r ) repair; (s) synthesis; (u) unspecified

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G30480 | (r ) | SSS(ph)PPGNVDGFSIGK | -0.09 | 7.93E-01 | 0.37 | 2.19E-01 | -0.14 | 7.48E-01 | 0.32 | 2.76E-01 |
| AT1G48620 | (s) | RVDAGGASSVAPPPPPPTNVES(ph)GGEEVAVK | -0.46 | 1.62E-01 | | 1.00E+00 | | 1.00E+00 | 1.26 | 4.04E-03 |
| AT1G77180 | (s) | ASGS(ph)PPVPVMHS(ph)PPRPVTVK | -0.05 | 6.11E-01 | -0.37 | 4.18E-01 | 0.96 | 1.37E-01 | 0.64 | 6.81E-01 |
| AT2G19490 | (s) | LIADEAADKETES(ph)ES(ph)EEEDSLR | -0.08 | 8.54E-01 | 0.47 | 3.91E-01 | -0.06 | 8.52E-01 | 0.50 | 2.33E-01 |
| AT2G25170 | (u) | KPVYNLDDS(ph)DDDDFVPK | -0.31 | 8.31E-01 | -0.13 | 5.58E-01 | 0.07 | 5.82E-01 | 0.25 | 8.03E-01 |
| AT2G25170 | (u) | KPVYNLDDS(ph)DDDDFVPKK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G30620 | (s) | (ac)S(ph)IEEENVPTTVDSGAADTTVK | -0.51 | 2.52E-01 | 0.30 | 6.34E-01 | -1.00 | 4.74E-02 | -0.19 | 6.93E-01 |
| AT2G42320 | (s) | GYT(ph)SDEELEELDSPLTSIVDK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.28 | 3.08E-01 |
| AT2G42320 | (s) | KGYT(ph)SDEELEELDSPLTSIVDK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 1.06 | 3.08E-01 |
| AT2G42320 | (s) | NVS(ph)M(ox)IQR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.78 | 1.36E-01 |
| AT2G47330 | (s) | AVDAGM(ox)LDYDS(ph)DDNPIVVDKR | -0.82 | 4.47E-02 | -0.61 | 7.31E-02 | 0.51 | 1.16E-01 | 0.72 | 7.17E-02 |
| AT3G14890 | (r ) | FFTPEEYFIPSSTS(ph)PGT | -0.62 | 2.30E-01 | -0.07 | 4.89E-01 | -0.02 | 3.53E-01 | 0.53 | 4.10E-01 |
| AT3G42170 | (u) | (ac)M(ox)EVYNDDTEM(ox)RS(ph)PETQPIK | 0.65 | 2.06E-01 | 0.88 | 2.75E-01 | -0.88 | 2.45E-01 | -0.66 | 1.82E-01 |
| AT3G60600 | (u) | (ac)S(ph)NIDLIGM(ox)SNR | | 1.00E+00 | 0.65 | 2.58E-01 | -0.94 | 1.05E-01 | | 1.00E+00 |
| AT3G60600 | (u) | ASVS(ph)DNGHGSEFSFER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT3G60600 | (u) | VTYVAPPRPPS(ph)PVHEGS(ph)EEGS(ph)SPR | 0.17 | 6.39E-01 | 0.39 | 7.07E-01 | 0.32 | 2.27E-01 | 0.53 | 2.48E-01 |
| AT5G55300 | (s) | EDGT(ph)DDDDDDDVPISK | 0.63 | 3.79E-01 | 0.37 | 6.27E-01 | -0.71 | 3.23E-01 | -0.97 | 1.88E-01 |
| AT5G55300 | (s) | IIKDES(ph)DDETPISSMFR | -1.27 | 2.66E-01 | | 1.00E+00 | | 1.00E+00 | 1.14 | 4.75E-01 |
| AT5G57160 | (s) | IGGDES(ph)DENDELDGNNNVSADAEEGNAAGR | -0.47 | 1.13E-01 | -0.28 | 8.09E-02 | 0.20 | 2.81E-01 | 0.39 | 1.91E-01 |

## others - gluconeogenesis / glyoxylate cycle.

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT4G15530 | GGM(ox)T(ph)SHAAVVAR | 1.10 | 6.15E-03 | -0.42 | 4.85E-01 | 0.47 | 3.38E-01 | -1.05 | 1.28E-02 |
| AT4G37870 | S(ph)APTTPINQNAAAAFAAVSEEER | -0.35 | 6.47E-01 | -1.64 | 5.64E-02 | 1.95 | 3.48E-02 | 0.66 | 4.25E-01 |
| AT4G37870 | S(ph)APTTPINQNAAAAFAAVSEEERQK | -0.72 | 3.77E-01 | | 1.00E+00 | | 1.00E+00 | 0.30 | 9.93E-01 |

## others - glycolysis.

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G07110 | SLS(ph)ASSFLIDTK | 0.38 | 9.99E-02 | 0.53 | 5.82E-02 | -0.01 | 9.65E-01 | 0.14 | 7.69E-01 |
| AT1G07110 | SLSASGS(ph)FR | 0.30 | 3.71E-01 | 0.84 | 2.10E-01 | -0.35 | 4.16E-01 | 0.20 | 2.54E-01 |
| AT1G12000 | DLTAVGS(ph)PENAPAK | | 1.00E+00 | 0.81 | 2.30E-03 | -0.34 | 8.10E-02 | | 1.00E+00 |
| AT1G13440 | AAS(ph)FNIIPSSTGAAK | 1.69 | 5.11E-02 | 0.31 | 9.27E-01 | -0.05 | 7.20E-01 | -1.43 | 3.79E-04 |
| AT1G13440 | LTGM(ox)S(ph)FR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G23190 | ATGAFILTAS(ph)HNPGGPTEDFGIK | -1.30 | 2.80E-01 | | 1.00E+00 | | 1.00E+00 | 0.25 | 8.18E-01 |
| AT1G53310 | M(ox)AS(ph)IDVHLR | | 1.00E+00 | 0.73 | 7.95E-02 | -0.69 | 6.75E-02 | | 1.00E+00 |
| AT3G14940 | M(ox)AS(ph)IDAQLR | 0.57 | 3.01E-01 | 0.51 | 2.01E-01 | 0.02 | 8.07E-01 | -0.04 | 9.32E-01 |
| AT3G14940 | MAS(ph)IDAQLR | 2.20 | 4.13E-03 | 0.70 | 2.92E-01 | 0.83 | 2.31E-01 | -0.67 | 1.61E-01 |
| AT3G14940 | S(ph)AQELVK | 1.60 | 2.54E-02 | 0.86 | 1.70E-01 | -1.10 | 1.12E-01 | -1.83 | 1.00E-02 |

## others - hormone metabolism.

(BR) brassinosteroids; (ET) ethylene; (IAA) auxin; (JA) jasmonate

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G23080 | (IAA) | PSNLTGAEIYS(ph)LNT(ph)TPR | -0.64 | 1.00E+00 | 0.34 | 9.52E-01 | 0.50 | 2.88E-01 | 1.48 | 7.36E-02 |
| AT1G68370 | (IAA) | AQGDESKGDGDS(ph)AGEEGGTENR | 1.16 | 4.76E-02 | 0.50 | 3.43E-01 | -0.16 | 8.55E-01 | -0.82 | 1.41E-01 |
| AT1G68370 | (IAA) | AQGDESKGDGDS(ph)AGEEGGTENRDK | 0.52 | 3.14E-02 | -0.08 | 6.63E-01 | 0.21 | 3.13E-01 | -0.40 | 8.84E-02 |
| AT1G70940 | (IAA) | M(ox)LIM(ox)EQFPETAASIVS(ph)FK | -0.42 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.53 | 1.00E+00 |
| AT1G70940 | (IAA) | PSNLTGAEIYS(ph)LST(ph)TPR | | 1.00E+00 | 0.23 | 4.71E-01 | | 1.00E+00 | | 1.00E+00 |
| AT3G04580 | (ET) | SILAGNAPELQHPNS(ph)NSILR | | 2.83E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G04580 | (ET) | SILAGNAPELQHPNSNS(ph)ILR | | 2.83E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G16460 | (JA) | (ac)S(ph)WDDGSHAK | | 1.00E+00 | -0.02 | 9.85E-01 | 1.22 | 2.38E-02 | | 1.00E+00 |
| AT3G19820 | (BR) | (ac)S(ph)DLQTPLVRPK | 0.49 | 2.07E-01 | 0.84 | 2.78E-02 | -0.10 | 7.40E-01 | 0.25 | 3.06E-01 |
| AT3G22850 | (IAA) | VGS(ph)VQNWSK | 0.23 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.13 | 3.24E-01 |
| AT4G27450 | (IAA) | VNS(ph)IPR | 0.06 | 2.17E-01 | -1.09 | 1.19E-01 | 0.30 | 5.48E-02 | -0.86 | 1.73E-01 |
| AT5G07120 | (IAA) | SPS(ph)SLSSDYIK | 0.07 | 8.19E-01 | -0.11 | 2.44E-01 | 0.18 | 2.08E-01 | 0.00 | 9.60E-01 |
| AT5G09410 | (IAA) | MQS(ph)FQR | 0.86 | 4.76E-02 | | 1.00E+00 | | 1.00E+00 | -0.38 | 1.54E-02 |
| AT5G43830 | (IAA) | TVANSPEALQS(ph)PHSSESAFALK | 0.10 | 5.30E-01 | -0.14 | 4.24E-01 | 0.48 | 3.43E-02 | 0.25 | 9.87E-01 |
| AT5G43830 | (IAA) | VDS(ph)SQNWAGHI | 0.97 | 3.81E-02 | 0.35 | 4.39E-01 | 0.34 | 4.36E-01 | -0.27 | 1.86E-01 |

## others - lipid metabolism.

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G13580 | LSEDVRS(ph)DS(ph)EGEDEHED | | 1.00E+00 | 0.76 | 1.00E+00 | 0.26 | 3.61E-02 | | 1.00E+00 |
| AT1G31480 | SADNLDEM(ox)EET(ph)DDEKDDR | -0.23 | 1.57E-01 | 0.05 | 9.20E-01 | -0.07 | 9.58E-01 | 0.21 | 2.53E-01 |
| AT1G31480 | SADNLDEMEET(ph)DDEKDDR | 0.94 | 1.72E-01 | 0.65 | 5.70E-01 | -0.27 | 8.27E-01 | -0.56 | 4.62E-02 |
| AT2G18730 | (ac)M(ox)DS(ph)PVSKTDASK | 0.69 | 4.13E-01 | -0.61 | 3.65E-01 | 0.24 | 8.63E-01 | -1.07 | 1.59E-01 |
| AT2G18730 | (ac)MDS(ph)PVSK | | 1.00E+00 | -0.28 | 1.00E+00 | 0.68 | 2.62E-02 | | 1.00E+00 |
| AT2G32260 | (ac)SNVIGDRTEDGLSTAAAASGSTAVQSS(ph)PPTDRPVR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G38040 | ELAAEES(ph)DGSVKEDDDDEDSSESGK | 0.34 | 8.19E-01 | 0.81 | 3.55E-01 | -0.12 | 7.07E-01 | 0.35 | 6.81E-01 |
| AT2G38040 | ELAAEES(ph)DGSVKEDDDDEDSSESGKSEMVNPSFA | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G42010 | EM(ox)VDGIDNS(ph)GTGSPSNANTPQALSR | | 1.00E+00 | 1.43 | 1.05E-01 | | 1.00E+00 | 0.26 | 3.12E-01 |
| AT2G42010 | EMVDGIDNS(ph)GTGSPSNANTPQALSR | 0.51 | 1.00E+00 | 0.12 | 1.05E-01 | 0.17 | 1.00E+00 | -0.22 | 3.12E-01 |
| AT2G42010 | EMVDGIDNSGT(ph)GSPSNANTPQALSR | | 1.00E+00 | -1.54 | 1.00E+00 | 1.01 | 3.37E-01 | | 1.00E+00 |
| AT3G03520 | SNPLSTS(ph)DPNSAQIFFGK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G03520 | VEETS(ph)SGGGSSASPIK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G03530 | WFASVPAS(ph)TQPNR | | 1.00E+00 | 0.53 | 1.61E-02 | 0.91 | 5.34E-03 | | 1.00E+00 |
| AT3G05420 | DIES(ph)EVEVSQEGR | 0.96 | 9.22E-03 | 0.37 | 6.00E-01 | -0.17 | 9.59E-01 | -0.75 | 2.62E-02 |
| AT3G05420 | TLEAPLPGS(ph)LSAVNNATTR | -0.10 | 9.78E-01 | -0.11 | 7.23E-01 | 0.37 | 2.97E-01 | 0.36 | 4.27E-01 |
| AT3G05630 | (ac)S(ph)TDKLLLPNGVK | 1.52 | 2.45E-02 | 1.42 | 8.57E-02 | -0.25 | 7.73E-01 | -0.35 | 3.01E-01 |
| AT3G05630 | HDSFS(ph)SAS(ph)PPQEIPLLLPQETDADFAGR | 0.14 | 3.62E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G07020 | TVVASIADETVAESSGTGNKS(ph)FSR | -1.75 | 4.67E-01 | 0.90 | 5.90E-03 | -1.01 | 1.47E-01 | 1.63 | 5.26E-01 |
| AT3G19260 | VGEDIRS(ph)DS(ph)EDDDD | 0.73 | 1.60E-01 | 0.74 | 1.63E-01 | -0.22 | 7.69E-01 | -0.21 | 6.78E-01 |
| AT4G00550 | DLGLSLNTPS(ph)PNTR | 0.76 | 1.38E-01 | 0.71 | 2.65E-01 | 0.15 | 8.07E-01 | 0.10 | 7.17E-01 |
| AT4G11850 | EVPVGTVSVYNS(ph)PR | 0.56 | 3.33E-02 | 0.69 | 3.26E-02 | -0.57 | 6.53E-02 | -0.44 | 6.45E-02 |
| AT4G11850 | LGGM(ox)LS(ph)GLGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G25970 | LSRPGS(ph)GSVSGLASQR | | 1.00E+00 | 0.42 | 3.43E-02 | 0.39 | 1.00E+00 | | 1.00E+00 |
| AT5G01220 | EDDES(ph)EIDAPLLDPESLSKPR | -0.18 | 7.28E-01 | -1.34 | 1.67E-02 | 2.27 | 5.90E-03 | 1.11 | 5.25E-02 |

## others - major CHO metabolism.

(d) degradation; (s) synthesis

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G35580 | (d) | HDGIHDS(ph)PR | 2.22 | 1.00E+00 | -0.15 | 3.64E-01 | -1.33 | 1.89E-01 | -3.70 | 1.00E+00 |
| AT1G35580 | (d) | KRS(ph)FDER | | 1.00E+00 | 0.81 | 4.07E-01 | -1.45 | 1.36E-01 | | 1.00E+00 |
| AT1G35580 | (d) | RS(ph)ASWPQL | | 1.00E+00 | 1.97 | 4.70E-02 | -0.87 | 3.88E-01 | | 1.00E+00 |
| AT1G35580 | (d) | S(ph)MSELST(ph)GYSR | | 1.00E+00 | | 1.11E-01 | 1.53 | 1.00E+00 | | 1.00E+00 |
| AT1G35580 | (d) | SAS(ph)WPQL | 0.39 | 1.87E-01 | 1.40 | 1.63E-05 | -0.68 | 9.14E-03 | 0.33 | 2.36E-01 |
| AT1G35580 | (d) | SVLDT(ph)PLSSAR | 0.63 | 1.76E-01 | 0.71 | 1.11E-01 | -0.73 | 9.51E-02 | -0.65 | 1.55E-01 |
| AT1G74910 | (s) | RVS(ph)SFEALQPATR | -0.82 | 2.64E-01 | 0.33 | 8.00E-01 | -0.54 | 3.81E-01 | 0.61 | 5.60E-01 |
| AT1G74910 | (s) | VSS(ph)FEALQPATR | 0.00 | 1.00E+00 | 0.89 | 6.81E-02 | -0.48 | 1.27E-01 | 0.40 | 1.04E-01 |
| AT3G23920 | (d) | AHGTDPS(ph)PPMS(ph)PILGATR | 0.98 | 1.01E-01 | | 4.23E-02 | 1.33 | 3.23E-01 | | 4.32E-01 |
| AT3G23920 | (d) | SGEM(ox)TDSSLLSIS(ph)PPSAR | 0.14 | 8.79E-01 | -0.33 | 1.00E+00 | -0.05 | 4.15E-01 | -0.52 | 2.30E-01 |
| AT4G10120 | (s) | VPEELTS(ph)DSLR | | 1.00E+00 | | 1.00E+00 | | 1.50E-02 | | 1.13E-01 |
| AT4G10120 | (s) | VPEELTS(ph)DSLRDVDDISLR | | 1.00E+00 | | 1.00E+00 | | 1.50E-02 | | 1.13E-01 |
| AT4G15210 | (d) | T(ph)SNSQLTLEDIADAAQPSGAFK | -0.44 | 4.05E-01 | -1.68 | 1.00E+00 | 1.64 | 6.68E-02 | 0.40 | 1.69E-01 |
| AT4G34860 | (d) | SLTELTGS(ph)PQLR | | 1.00E+00 | 0.92 | 3.40E-03 | -0.86 | 4.89E-03 | | 1.00E+00 |
| AT5G11110 | (s) | S(ph)GSNNGVDTNLDAEDR | 0.20 | 3.06E-01 | | 1.00E+00 | | 1.00E+00 | -0.35 | 2.30E-01 |
| AT5G16150 | (d) | AQASS(ph)DGDEEEAIPLR | | 1.00E+00 | -0.03 | 3.84E-01 | -0.09 | 1.00E+00 | | 1.00E+00 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT5G16150 | (d) | AQASS(ph)DGDEEEAIPLRSEGK | | 1.00E+00 | | 3.84E-01 | | 1.00E+00 | | 1.00E+00 |
| AT5G20280 | (s) | DIQDIS(ph)LNLK | 1.30 | 5.80E-04 | 0.84 | 3.27E-03 | 0.52 | 3.64E-02 | 0.06 | 7.83E-01 |
| AT5G20280 | (s) | FS(ph)FDGSGNDNYM(ox)NQEGSSM(ox)DR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G20280 | (s) | FSFDGS(ph)GNDNYM(ox)NQEGSSM(ox)DRK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G20280 | (s) | HPQWQSDDGGDNS(ph)EPES(ph)PSDSLR | 1.14 | 8.58E-03 | 0.64 | 4.67E-02 | 0.38 | 6.82E-02 | -0.12 | 2.11E-01 |
| AT5G20280 | (s) | INS(ph)AESM(ox)ELWASQQK | 0.51 | 4.81E-01 | 0.93 | 1.96E-01 | -0.71 | 1.27E-01 | -0.28 | 4.99E-01 |
| AT5G20280 | (s) | S(ph)SPSLLLR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |

## others - minor CHO metabolism.

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G06410 | S(ph)YTNLLDLASGNFPVM(ox)GR | -0.43 | 3.68E-01 | | 1.00E+00 | | 1.00E+00 | -0.22 | 6.24E-01 |
| AT1G06410 | VM(ox)TVPGNVSEFDEDQAYSVSS(ph)DNPSSVSSDR | | 3.68E-01 | | 1.00E+00 | | 1.00E+00 | 1.21 | 1.00E+00 |
| AT1G34120 | IKSHS(ph)DPPS(ph)PSK | -0.16 | 8.56E-01 | 0.27 | 2.20E-01 | 0.82 | 6.42E-01 | 1.25 | 3.58E-01 |
| AT1G34120 | S(ph)QEFDPISSGVTNPR | 0.58 | 2.17E-01 | 0.63 | 3.33E-01 | -0.28 | 7.06E-01 | -0.23 | 6.17E-01 |
| AT1G70290 | VLSLSPS(ph)FR | | 1.00E+00 | 0.37 | 4.95E-02 | -0.22 | 2.42E-01 | | 1.00E+00 |
| AT1G71710 | SYS(ph)DPPS(ph)PGR | 0.57 | 1.04E-01 | -1.08 | 5.84E-02 | 0.91 | 6.68E-02 | -0.74 | 8.42E-02 |
| AT4G03550 | ASS(ph)SVSTLYK | -0.21 | 1.70E-01 | 0.79 | 1.26E-01 | -1.25 | 3.47E-02 | -0.24 | 1.40E-01 |
| AT5G40390 | SDS(ph)GINGVDFTEK | 0.11 | 7.68E-01 | 1.35 | 4.16E-03 | -0.82 | 4.24E-02 | 0.41 | 2.64E-01 |
| AT5G57330 | LELSAVPS(ph)SYSSGQLDPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |

## others - metal handling.

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G30470 | ASGIEPTES(ph)SPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G30470 | TPGVPGDETTEKLPDESGVEPTENS(ph)PK | | 1.00E+00 | 0.62 | 1.00E+00 | | 1.00E+00 | -0.42 | 1.00E+00 |
| AT5G19140 | (ac)M(ox)LGIFSGAIVSPPEELVAAGSRT(ph)PSPK | | 1.00E+00 | | 9.18E-02 | | 1.00E+00 | -0.30 | 1.35E-01 |

## others - misc.

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G07990 | T(ph)YNANTSSSDDDEVVVGEEDDDLTGNPK | | 4.22E-02 | 0.74 | 1.00E+00 | | 1.00E+00 | | 4.89E-01 |
| AT1G10290 | AAAASSYSDNSGTESS(ph)PR | 0.26 | 7.13E-03 | 0.23 | 7.61E-01 | -0.41 | 3.15E-01 | -0.44 | 2.24E-01 |
| AT1G10290 | ATS(ph)PQPDGPTAGGSLK | 0.00 | 9.92E-01 | -0.86 | 1.42E-02 | 1.05 | 3.96E-03 | 0.19 | 3.56E-01 |
| AT1G16610 | GRS(ph)PPPPPSK | 0.98 | 2.23E-01 | -0.31 | 2.09E-01 | 0.27 | 2.03E-01 | -1.02 | 2.28E-01 |
| AT1G16610 | GRS(ph)PSS(ph)PPPRR | -2.93 | 5.53E-04 | -0.95 | 1.08E-01 | 0.07 | 9.39E-01 | 2.05 | 2.83E-03 |
| AT1G16610 | IRGS(ph)PVR | -1.12 | 8.95E-02 | -0.46 | 4.73E-01 | -0.06 | 7.76E-01 | 0.60 | 3.39E-01 |
| AT1G16610 | RGRS(ph)PPPPPSK | -1.24 | 7.17E-01 | -0.75 | 2.93E-01 | 0.40 | 4.01E-01 | 0.89 | 8.77E-01 |
| AT1G16610 | S(ph)PLPLR | 0.20 | 5.97E-01 | 0.50 | 2.81E-01 | -0.17 | 7.21E-01 | 0.13 | 8.54E-01 |
| AT1G16610 | S(ph)PPRRS(ph)PIR | 1.63 | 7.25E-03 | -0.68 | 1.07E-01 | 0.50 | 1.90E-01 | -1.82 | 1.73E-02 |
| AT1G16610 | VSS(ph)PPKPVSAAPK | 0.61 | 2.06E-02 | -0.16 | 8.04E-01 | -0.24 | 8.00E-01 | -1.02 | 7.53E-03 |
| AT1G16610 | YRS(ph)PPRGS(ph)PR | 1.02 | 1.36E-03 | -0.10 | 8.10E-01 | 0.26 | 2.35E-01 | -0.87 | 9.31E-03 |
| AT1G33990 | TLS(ph)DPFSNGK | 0.30 | 3.23E-01 | 0.72 | 4.03E-02 | -0.35 | 2.77E-01 | 0.06 | 9.00E-01 |
| AT1G59610 | AAAASSWSDNSGTESS(ph)PR | | 1.00E+00 | 0.85 | 1.75E-01 | 0.55 | 2.99E-02 | | 1.00E+00 |
| AT1G59610 | ATS(ph)PQPDGPSSTGGSLK | 0.30 | 3.56E-01 | -0.43 | 9.64E-02 | 0.48 | 6.61E-02 | -0.24 | 4.88E-01 |
| AT1G59610 | QLS(ph)IHDNR | | 1.00E+00 | -0.18 | 1.00E+00 | 0.71 | 1.35E-02 | | 1.00E+00 |
| AT1G59610 | QSLS(ph)EGSLDK | 1.33 | 9.26E-02 | -0.42 | 7.54E-01 | 0.84 | 5.03E-01 | -0.92 | 1.78E-01 |
| AT1G59610 | RYS(ph)DPAQNGEDSSGSGGSSR | 0.63 | 4.93E-01 | | 1.00E+00 | | 1.00E+00 | -0.32 | 9.75E-01 |
| AT2G01180 | GVPVTSS(ph)QNGDALR | 0.04 | 1.00E+00 | -0.59 | 6.70E-02 | 0.21 | 1.00E+00 | -0.43 | 1.14E-01 |
| AT2G28890 | GFLS(ph)GPIER | | 1.00E+00 | -1.02 | 1.16E-02 | 0.09 | 1.00E+00 | | 1.00E+00 |
| AT2G34410 | VHS(ph)DNNLVELGEVK | | 1.00E+00 | -0.69 | 3.54E-01 | 1.67 | 2.64E-01 | | 1.00E+00 |
| AT3G26180 | S(ph)PVDLSK | 0.38 | 2.11E-01 | 0.36 | 2.18E-01 | -0.08 | 6.77E-01 | -0.10 | 7.10E-02 |
| AT3G45190 | IPNGSSSSEGEIS(ph)PR | 0.02 | 4.27E-01 | -0.59 | 1.00E+00 | -0.43 | 1.00E+00 | -1.04 | 3.29E-02 |
| AT3G45190 | S(ph)PPVPSLFGK | 3.44 | 4.75E-04 | 0.73 | 8.15E-03 | -0.44 | 5.97E-02 | -3.15 | 7.26E-05 |
| AT3G45190 | T(ph)RDSDDDEVHDR | -1.30 | 4.75E-04 | 0.26 | 1.00E+00 | 0.18 | 1.00E+00 | 1.74 | 7.26E-05 |
| AT3G45190 | TRDS(ph)DDDEVHDR | -4.83 | 9.04E-02 | 1.44 | 2.38E-01 | -2.42 | 2.76E-01 | 3.85 | 1.00E+00 |
| AT4G10020 | VPAS(ph)PPR | 1.24 | 4.31E-02 | 1.58 | 1.00E+00 | -1.18 | 1.50E-02 | -0.84 | 1.13E-01 |
| AT4G33650 | TIEEVSGETPPS(ph)T(ph)PPSSSTPSPSSSTTNAAPLGSSVIPIVNK | | 1.00E+00 | | 9.72E-02 | | 2.33E-02 | | 1.00E+00 |
| AT5G15070 | QGS(ph)GIIGTFGQSEELR | 0.13 | 4.33E-01 | -0.61 | 1.86E-02 | 1.05 | 5.31E-04 | 0.31 | 4.57E-01 |

## others - N-metabolism.

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G37130 | SVS(ph)TPFMNTTAK | 0.41 | 1.93E-01 | -0.26 | 3.81E-01 | 0.35 | 2.42E-01 | -0.33 | 2.94E-01 |
| AT3G53180 | LNIDTDSSSPQNIIS(ph)PK | 0.32 | 1.46E-01 | 0.42 | 1.89E-01 | 0.36 | 1.88E-01 | 0.46 | 1.63E-01 |
| AT5G67220 | DGQIEETVDTAPASLGS(ph)PSR | 0.06 | 7.15E-01 | 0.55 | 5.30E-02 | -0.13 | 7.54E-01 | 0.36 | 1.25E-01 |

## others - nucleotide metabolism.

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G26190 | LSLDEDLVSS(ph)SSPK | | 1.00E+00 | 0.61 | 5.50E-02 | -0.65 | 4.17E-02 | | 1.00E+00 |
| AT1G68720 | VLPQEAPSLHQVEVGQT(ph)SPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G73980 | APAS(ph)PATLPNQGFITQLSDQISTLNER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G73980 | LSLDDDTVS(ph)SPKEALSR | -0.60 | 1.83E-01 | 0.80 | 1.88E-01 | -0.64 | 1.80E-01 | 0.76 | 1.92E-01 |
| AT1G73980 | LSLDDDTVSS(ph)PK | -0.51 | 3.13E-01 | 0.86 | 4.63E-01 | -1.25 | 9.64E-02 | 0.12 | 9.14E-01 |
| AT2G38280 | EVISDPS(ph)TPKPNTEPFAHYPQGK | | 1.00E+00 | 0.71 | 1.00E+00 | | 1.00E+00 | 2.34 | 1.00E+00 |
| AT2G38280 | KVNDQYGRS(ph)PASLPDATPFTDGGGGGGGDTGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G38280 | S(ph)PASLPDATPFTDGGGGGGGDTGR | 0.36 | 3.61E-01 | 1.20 | 3.96E-02 | -1.00 | 7.40E-02 | -0.16 | 5.69E-01 |
| AT2G38280 | SHS(ph)VSGDLHGVQPDPIAADILR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G38280 | TGS(ph)FVRPIS(ph)PK | -0.14 | 6.22E-01 | 0.02 | 9.45E-01 | -0.67 | 8.13E-02 | -0.51 | 3.54E-01 |
| AT2G38280 | VNDQYGRS(ph)PASLPDATPFTDGGGGGGGDTGR | -0.96 | 5.21E-02 | -0.11 | 6.55E-01 | -0.12 | 8.48E-01 | 0.72 | 1.79E-01 |
| AT3G27190 | LDGLLSS(ph)PSK | 1.00 | 1.92E-02 | 0.83 | 4.81E-02 | -0.34 | 2.62E-01 | -0.51 | 5.46E-03 |
| AT5G18280 | SPSSTELLESGNHS(ph)PTSDSVDGGK | 0.81 | 4.37E-01 | 0.36 | 6.10E-02 | 0.28 | 4.78E-01 | -0.17 | 8.03E-01 |
| AT5G40870 | FDGLLS(ph)SSPPNSSVVSSLR | 0.54 | 1.23E-01 | -0.50 | 4.40E-01 | 0.45 | 1.60E-01 | -0.59 | 4.02E-01 |

## others - protein.

(a) amino acid activation; (f) folding; (g) glycosylation; (s) synthesis; (t) targeting

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G01100 | (s) | DEPAEES(ph)DGDLGFGLFD | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G01100 | (s) | KDEPAEES(ph)DGDLGFGLFD | -0.62 | 4.50E-02 | -0.86 | 2.80E-03 | 1.33 | 7.79E-05 | 1.09 | 1.49E-03 |
| AT1G01100 | (s) | KKDEPAEES(ph)DGDLGFGLFD | -2.11 | 9.53E-03 | -2.06 | 1.08E-03 | 1.88 | 9.08E-04 | 1.92 | 8.10E-03 |
| AT1G12920 | (s) | TFDELS(ph)DTEVYEDSD | 0.53 | 3.89E-01 | 0.73 | 2.57E-01 | -0.15 | 9.68E-01 | 0.05 | 8.29E-01 |
| AT1G12930 | (t) | LVDEDAESS(ph)GRQSPATYTR | -0.88 | 1.00E+00 | | 1.00E+00 | -1.71 | 2.38E-02 | | 1.00E+00 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G15930 | (s) | (ac)S(ph)GDEAAPVVVPPPVAEPAAIPEDM(ox)DLM(ox)TALELTLRK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G18950 | (a) | GSSSDIVPDRS(ph)PADDVAPVTDTK | -0.18 | 6.68E-01 | 0.34 | 1.04E-01 | 0.08 | 8.59E-02 | 0.60 | 1.31E-01 |
| AT1G25260 | (s) | EDLS(ph)DVETS | 0.36 | 3.03E-01 | 0.80 | 3.43E-02 | -0.43 | 2.21E-01 | 0.02 | 9.76E-01 |
| AT1G25260 | (s) | EDLSELYREDLS(ph)DVETS | -0.64 | 1.07E-01 | -1.01 | 4.07E-02 | 1.15 | 5.57E-03 | 0.78 | 3.83E-02 |
| AT1G26170 | (t) | SSIDGAEDSYDGRYDS(ph)DGEEK | 0.94 | 2.71E-02 | -0.39 | 1.22E-01 | -0.24 | 1.70E-01 | -1.57 | 9.81E-03 |
| AT1G26630 | (s) | (ac)S(ph)DDEHHFEASESGASK | 0.34 | 7.80E-01 | 0.11 | 6.66E-01 | 0.15 | 5.26E-01 | -0.08 | 9.31E-01 |
| AT1G30230 | (s) | ISGVSAEGSGVIVEGSAPITEEAVAT(ph)PPAADSK | -0.23 | 7.95E-01 | | 1.00E+00 | | 1.00E+00 | 0.77 | 1.41E-01 |
| AT1G31970 | (s) | ITFDNS(ph)DDED | | 1.00E+00 | 0.06 | 7.99E-01 | -0.35 | 3.66E-01 | | 1.00E+00 |
| AT1G36730 | (s) | SLSDENDQADS(ph)EEDDDDVQWQTDTSR | 0.14 | 5.93E-01 | 0.13 | 6.39E-01 | 0.34 | 3.01E-01 | 0.33 | 3.30E-01 |
| AT1G48920 | (s) | AASSSDSSDEDS(ph)DEESEDEKPAQK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G48920 | (s) | GFDASLS(ph)EDDIK | 1.06 | 1.10E-01 | 1.36 | 1.09E-01 | -0.66 | 1.18E-01 | -0.36 | 3.47E-01 |
| AT1G48920 | (s) | GFDASLS(ph)EDDIKNTLR | -1.25 | 1.48E-01 | -2.23 | 4.65E-03 | 1.53 | 4.97E-02 | 0.55 | 6.88E-01 |
| AT1G64790 | (s) | ALLEGGS(ph)DDEGASTEAQGR | 0.61 | 3.36E-02 | 0.12 | 8.36E-01 | -0.27 | 6.44E-01 | -0.77 | 1.94E-02 |
| AT1G69410 | (s) | (ac)S(ph)DDEHHFESSDAGASK | 0.94 | 8.78E-03 | 0.32 | 3.01E-01 | 0.12 | 5.63E-01 | -0.51 | 9.16E-02 |
| AT1G72160 | (t) | KSM(ox)IPQNLGS(ph)FKEESSK | -1.44 | 2.19E-01 | 0.89 | 2.98E-01 | -1.34 | 1.67E-01 | 0.99 | 3.60E-01 |
| AT1G72160 | (t) | LPS(ph)PSLTPSEVSESTQDALPTETETLEK | -1.03 | 3.94E-01 | | 1.00E+00 | | 1.00E+00 | 2.69 | 1.18E-01 |
| AT1G72160 | (t) | SM(ox)IPQNLGS(ph)FKEESSK | -0.22 | 4.12E-01 | 0.14 | 4.33E-01 | -0.14 | 4.70E-01 | 0.22 | 3.77E-01 |
| AT1G72340 | (s) | SPS(ph)FILDER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G72340 | (s) | SSNS(ph)PPM(ox)ADTTR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G76810 | (s) | AHDS(ph)EPEAEKPTAK | 0.68 | 3.06E-01 | | 1.00E+00 | | 1.00E+00 | -0.96 | 2.06E-01 |
| AT1G76810 | (s) | S(ph)WGTVDLNLK | 0.64 | 1.16E-01 | -0.26 | 4.14E-01 | 1.08 | 1.28E-02 | 0.17 | 7.16E-01 |
| AT1G80680 | (t) | LM(ox)YAAS(ph)DNEEDVM(ox)QDVKEDSAK | -0.95 | 1.60E-01 | -0.03 | 9.65E-01 | -0.61 | 3.27E-01 | 0.31 | 6.12E-01 |
| AT2G17980 | (t) | SLNASFAAT(ph)SANSASR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G18240 | (t) | (ac)M(ox)EDEPGS(ph)ENEADTIVASPLAK | 0.05 | 3.99E-02 | 0.75 | 3.97E-02 | -0.69 | 5.32E-02 | 0.01 | 9.15E-01 |
| AT2G27720 | (s) | EEKEES(ph)DDDM(ox)GFSLFE | 0.49 | 8.80E-02 | 0.66 | 3.04E-02 | 0.12 | 5.70E-01 | 0.30 | 4.26E-01 |
| AT2G27720 | (s) | EES(ph)DDDM(ox)GFSLFE | 0.02 | 7.27E-01 | 1.75 | 3.61E-02 | -0.58 | 3.57E-01 | 1.15 | 2.74E-01 |
| AT2G27720 | (s) | KEEKEES(ph)DDDMGFSLFE | -1.79 | 3.00E-02 | -1.56 | 3.79E-03 | 1.71 | 1.38E-03 | 1.93 | 1.17E-02 |
| AT2G27720 | (s) | LASVPSGGGGGVAVAS(ph)ATSGGGGGGGAPAAESK | 0.22 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G28800 | (t) | DTVELVEESQSESEEGS(ph)DDEEEEAR | -0.04 | 9.57E-01 | 0.25 | 4.23E-01 | 0.20 | 5.59E-01 | 0.49 | 2.00E-01 |
| AT2G34970 | (s) | DKLSEITQAIDDDDT(ph)DDESVVPTSGELK | -2.19 | 2.32E-01 | | 1.00E+00 | | 1.00E+00 | 1.01 | 8.73E-01 |
| AT2G34970 | (s) | LSEITQAIDDDDT(ph)DDESVVPTSGELK | -0.31 | 4.20E-01 | 0.10 | 3.09E-01 | 0.46 | 1.80E-01 | 0.87 | 7.14E-02 |
| AT2G34970 | (s) | VSLLQQPTTEDS(ph)DEELEYADSSSGTADHLSGLNLQM(ox)ESK | | 1.00E+00 | | 3.09E-01 | | 1.80E-01 | 0.75 | 1.00E+00 |
| AT2G41840 | (s) | ALS(ph)TSKPDPVVEDQA | -0.24 | 4.23E-01 | 0.29 | 1.91E-01 | -0.37 | 2.06E-01 | 0.17 | 4.52E-01 |
| AT2G45140 | (t) | AS(ph)VSDNGNASDFTAAPR | -0.06 | 4.07E-01 | -0.04 | 3.55E-01 | -0.53 | 5.65E-01 | -0.50 | 1.00E+00 |
| AT2G45140 | (t) | FS(ph)ADRVDAQDNSSEAR | 0.57 | 3.41E-01 | 0.32 | 7.62E-01 | -0.70 | 5.28E-01 | -0.94 | 2.09E-01 |
| AT2G45140 | (t) | VVYVAPPRPPS(ph)PVR | 0.23 | 4.92E-01 | 0.25 | 9.97E-01 | 0.69 | 2.45E-01 | 0.71 | 5.14E-01 |
| AT2G45140 | (t) | VVYVAPPRPPS(ph)PVREGSEEGS(ph)SPR | -0.42 | 6.19E-01 | 0.07 | 4.16E-01 | 0.28 | 6.48E-01 | 0.78 | 9.83E-02 |
| AT3G06700 | (s) | KHNVKAGENAS(ph)AEE | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G09200 | (s) | EES(ph)DEEDYGGDFGLFDEE | -0.57 | 1.08E-01 | -0.48 | 1.08E-01 | 1.20 | 4.07E-03 | 1.29 | 2.27E-03 |
| AT3G09200 | (s) | VEEKEES(ph)DEEDYGGDFGLFDEE | -0.41 | 4.03E-01 | -2.06 | 1.15E-05 | 2.22 | 4.67E-06 | 0.57 | 2.20E-01 |
| AT3G11250 | (s) | KEES(ph)DEEDYEGGFGLFDEE | -0.40 | 3.22E-01 | -1.82 | 3.63E-05 | 2.17 | 4.84E-06 | 0.75 | 5.95E-02 |
| AT3G11250 | (s) | VEEKKEES(ph)DEEDYEGGFGLFDEE | -0.58 | 1.61E-01 | -1.36 | 6.35E-02 | 0.20 | 1.00E+00 | -0.58 | 2.87E-01 |
| AT3G12390 | (s) | IDLDKPEVEDDDDNEDDDS(ph)DDDDKDDDEADGLDGEAGGK | -0.30 | 3.61E-01 | 0.35 | 3.37E-01 | 0.22 | 6.30E-01 | 0.86 | 1.39E-02 |
| AT3G18480 | (t) | KYVEDLESGFSS(ph)DVESK | -0.46 | 4.74E-01 | | 1.00E+00 | | 1.00E+00 | 1.86 | 1.99E-01 |
| AT3G18480 | (t) | YVEDLESGFSS(ph)DVESK | -0.01 | 9.64E-01 | -0.09 | 5.22E-01 | -0.07 | 7.97E-01 | -0.15 | 4.40E-01 |
| AT3G19770 | (t) | AES(ph)ISDLENK | 1.00 | 9.41E-02 | | 1.00E+00 | | 1.00E+00 | -0.49 | 2.02E-01 |
| AT3G19770 | (t) | S(ph)SDSLSGTNELLNINSETPM(ox)K | -0.34 | 5.13E-01 | 0.14 | 1.43E-01 | 0.42 | 3.52E-01 | 0.90 | 1.75E-01 |
| AT3G19770 | (t) | S(ph)SDSLSGTNELLNINSETPM(ox)KK | -0.45 | 5.27E-01 | 0.50 | 3.58E-01 | 0.18 | 6.01E-01 | 1.14 | 6.08E-02 |
| AT3G20050 | (f) | (ac)S(ph)ISAQNPDISGDR | | 1.00E+00 | | 1.00E+00 | | 2.42E-01 | | 1.00E+00 |
| AT3G26618 | (s) | TFDELS(ph)DGEVYEDSD | 0.40 | 5.69E-01 | 0.79 | 3.60E-01 | -0.17 | 8.44E-01 | 0.23 | 6.59E-01 |
| AT3G27530 | (t) | LLEDIGDESEAQAES(ph)EED | 0.30 | 3.51E-01 | 1.08 | 3.89E-03 | -0.48 | 1.51E-01 | 0.30 | 2.91E-01 |
| AT3G49010 | (s) | AGDS(ph)TPEELANATQVQGDYLPIVR | -0.25 | 4.59E-01 | -0.47 | 5.84E-01 | 0.56 | 2.91E-01 | 0.34 | 1.82E-01 |
| AT3G49010 | (s) | VKAGDS(ph)TPEELANATQVQGDYLPIVR | | 1.00E+00 | | 1.59E-02 | | 2.26E-02 | 1.38 | 1.00E+00 |
| AT3G56150 | (s) | FFTQVGSESEDES(ph)DYEVEVNEVQNDDVNNR | | 1.00E+00 | | 3.40E-01 | | 2.59E-01 | | 1.00E+00 |
| AT3G56150 | (s) | YLQS(ph)GS(ph)EDDDDTDTK | 0.28 | 5.54E-01 | 0.46 | 3.86E-01 | -0.31 | 5.22E-01 | -0.13 | 7.27E-01 |
| AT3G56150 | (s) | YLQS(ph)GS(ph)EDDDDTDTKR | 0.06 | 6.20E-01 | -0.04 | 7.88E-01 | -0.06 | 5.31E-01 | -0.16 | 1.66E-01 |
| AT3G57150 | (s) | DKKEEVIEEVAS(ph)PK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G57150 | (s) | DTEAAVDAEDES(ph)AAEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G57150 | (s) | DTEAAVDAEDES(ph)AAEKSEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G57150 | (s) | EEVIEEVAS(ph)PK | 0.76 | 1.24E-01 | 0.49 | 3.55E-01 | 0.08 | 6.20E-01 | -0.19 | 7.02E-01 |
| AT3G57150 | (s) | HDDSSDS(ph)PAPVTTKK | 1.97 | 2.11E-02 | -2.07 | 1.00E+00 | 1.50 | 3.59E-02 | -2.54 | 1.00E+00 |
| AT3G57150 | (s) | KHDDSSDS(ph)PAPVTTK | | 1.00E+00 | 0.86 | 2.76E-02 | 0.47 | 1.33E-01 | | 1.00E+00 |
| AT3G57150 | (s) | SKDTEAAVDAEDES(ph)AAEK | -0.01 | 4.28E-01 | | 1.00E+00 | | 1.00E+00 | 0.28 | 4.52E-01 |
| AT3G57490 | (s) | ISEVVVDKS(ph)VE | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G60240 | (s) | QVLQGPSATVNS(ph)PR | 0.68 | 3.03E-02 | 0.66 | 3.61E-02 | -0.70 | 2.71E-02 | -0.72 | 2.35E-02 |
| AT4G00100 | (s) | S(ph)SPSWLK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G00810 | (s) | EEKKDEPAEES(ph)DGDLGFGLFD | -1.48 | 2.20E-01 | -1.13 | 4.12E-01 | 1.28 | 1.35E-01 | 1.63 | 6.61E-02 |
| AT4G02510 | (t) | ELDSSSEAVSGNSDKVGADDLS(ph)DSEK | 0.33 | 9.86E-01 | 1.44 | 1.53E-01 | -1.38 | 9.11E-02 | -0.26 | 6.66E-01 |
| AT4G02510 | (t) | IDGQIVT(ph)DSDEDVDTEDEGEEK | -0.10 | 8.78E-01 | 1.19 | 9.57E-02 | -0.40 | 7.11E-01 | 0.89 | 1.72E-01 |
| AT4G02510 | (t) | KVVEGDS(ph)AEEDENKLPVEDIVSSR | -0.89 | 4.64E-01 | | 1.00E+00 | | 1.00E+00 | 0.95 | 2.37E-01 |
| AT4G02510 | (t) | VDGS(ph)ES(ph)EEETEEM(ox)IFGSSEAAK | -0.88 | 5.03E-02 | 0.71 | 1.28E-01 | -0.73 | 9.55E-02 | 0.86 | 6.80E-02 |
| AT4G02510 | (t) | VGADDLS(ph)DSEK | 0.85 | 3.11E-01 | 1.06 | 3.39E-01 | 0.01 | 9.75E-01 | 0.22 | 7.94E-02 |
| AT4G02510 | (t) | VGADDLS(ph)DSEKEKPNLVGDGK | 0.25 | 8.36E-01 | 0.73 | 1.95E-01 | -0.23 | 7.65E-01 | 0.25 | 5.34E-01 |
| AT4G02510 | (t) | VVEGDS(ph)AEEDENKLPVEDIVSSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.58 | 5.29E-01 |
| AT4G20980 | (s) | NLSNPSARPNT(ph)GSK | 0.93 | 9.58E-02 | -0.51 | 9.08E-02 | 0.53 | 1.35E-01 | -0.91 | 9.62E-02 |
| AT4G25340 | (f) | QIVAIEGAHVPVLES(ph)EDEDEDGLPIPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.94 | 1.00E+00 |
| AT4G31180 | (a) | DPQRLS(ph)P | 0.15 | 3.34E-01 | | 1.00E+00 | | 1.00E+00 | -0.18 | 3.87E-01 |
| AT4G31700 | (s) | DRRS(ph)ESLAK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G31700 | (s) | LS(ph)SAAAKPSVTA | 0.47 | 2.23E-02 | 0.56 | 1.35E-02 | -0.24 | 1.44E-01 | -0.15 | 2.89E-01 |
| AT4G31700 | (s) | SRLS(ph)SAAAKPSVTA | -0.52 | 1.69E-01 | 0.90 | 6.58E-02 | -1.35 | 9.21E-03 | 0.07 | 8.47E-01 |
| AT5G06140 | (t) | NISGS(ph)M(ox)QSPR | -0.34 | 7.49E-01 | 2.02 | 1.42E-02 | -1.54 | 2.62E-02 | 0.83 | 9.94E-01 |
| AT5G08180 | (s) | GS(ph)DTEAEKSIQK | | 1.00E+00 | -0.90 | 1.64E-01 | -0.58 | 1.00E+00 | | 1.00E+00 |
| AT5G10360 | (s) | LS(ph)SAPAKPVAA | 1.23 | 6.05E-02 | 1.18 | 5.79E-02 | -0.50 | 4.15E-01 | -0.55 | 3.68E-01 |
| AT5G10360 | (s) | S(ph)RLS(ph)SAPAKPVAA | 0.19 | 6.25E-01 | 0.90 | 6.37E-02 | -0.85 | 7.64E-02 | -0.14 | 7.37E-01 |
| AT5G14050 | (s) | KQYEDVEDEEEIGS(ph)DDDLTR | 0.14 | 8.50E-01 | -0.39 | 4.51E-01 | 0.61 | 2.59E-01 | 0.09 | 8.26E-01 |
| AT5G14050 | (s) | QIPDYEDDGDDDEELS(ph)DEENGQVVAIR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G14050 | (s) | QYEDVEDEEEIGS(ph)DDDLTR | 0.43 | 1.49E-01 | -0.60 | 1.88E-02 | 1.17 | 3.96E-03 | 0.13 | 9.31E-01 |
| AT5G15200 | (s) | DLLTLDEKS(ph)PR | | 1.00E+00 | 0.43 | 6.14E-01 | 0.38 | 6.07E-01 | | 1.00E+00 |
| AT5G19980 | (g) | VS(ph)EKDSEKGEEDEELTQLVPGK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G27640 | (s) | DGEVS(ph)DVEEDEYEAK | 1.06 | 1.03E-02 | 0.38 | 6.65E-01 | 0.04 | 8.82E-01 | -0.63 | 3.63E-02 |
| AT5G27640 | (s) | QNLRDGEVS(ph)DVEEDEYEAK | -0.24 | 4.60E-01 | -0.58 | 2.21E-01 | 0.39 | 5.94E-01 | 0.04 | 9.38E-01 |
| AT5G38640 | (s) | DFPDGSTTAS(ph)PGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G38640 | (s) | VAVAGAAASAVS(ph)PSSFSYSSR | | 1.00E+00 | -0.97 | 1.00E+00 | | 1.00E+00 | -0.69 | 2.23E-01 |
| AT5G40930 | (t) | APELHTGGTAGPSS(ph)NSAK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G45775 | (s) | VLEQLSGQT(ph)PVFSK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G52280 | (t) | NS(ph)PENSFQR | 1.01 | 1.92E-02 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G57290 | (s) | KEES(ph)EEEEGDFGFDLFG | | 1.00E+00 | | 8.09E-02 | | 2.81E-01 | 1.31 | 1.00E+00 |
| AT5G57290 | (s) | KKEES(ph)EEEEGDFGFDLFG | -4.04 | 7.73E-02 | | 1.00E+00 | | 1.00E+00 | 2.88 | 1.09E-01 |

## others - photosynthesis

(CC) calvin cycle; (LR) light reaction

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G68830 | (LR) | T(ph)VTET(ph)IDEISDGRK | | 1.00E+00 | 0.10 | 7.93E-01 | -1.12 | 3.18E-01 | | 1.00E+00 |
| AT2G28000 | (CC) | NVVLDEFGS(ph)PK | | 1.00E+00 | 0.69 | 9.43E-01 | -0.54 | 8.21E-01 | | 1.00E+00 |
| AT2G34420 | (LR) | GPSGS(ph)PWYGSDR | | 1.00E+00 | | 3.54E-01 | | 2.64E-01 | | 1.00E+00 |
| AT2G39730 | (CC) | GLAYDT(ph)SDDQQDITR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G46820 | (LR) | AT(ph)TEVGEAPATTTEAETTELPEIVK | -1.04 | 1.82E-01 | -1.04 | 1.24E-01 | -0.24 | 6.88E-01 | -0.24 | 4.15E-01 |
| AT3G61470 | (LR) | WADIIKPGS(ph)VNTDPVFPNNK | | 1.81E-01 | | 1.00E+00 | | 1.00E+00 | -0.50 | 4.13E-01 |
| AT4G02630 | (LR) | NSGGGGGGIEQGRS(ph)PR | 1.07 | 9.19E-02 | 0.54 | 5.66E-01 | -0.25 | 8.93E-01 | -0.78 | 2.14E-01 |
| AT5G01530 | (LR) | NLAGDVIGT(ph)RTEAADAK | 0.70 | 1.01E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| atcg00020 | (LR) | (ac)T(ph)AILER | 0.22 | 7.84E-01 | 1.26 | 8.20E-02 | -0.92 | 1.89E-01 | 0.12 | 8.25E-01 |
| atcg00020 | (LR) | (ac)T(ph)AILERR | 0.07 | 7.98E-01 | -0.57 | 3.18E-01 | -0.11 | 5.61E-01 | -0.75 | 2.54E-01 |
| atcg00270 | (LR) | (ac)T(ph)IALGK | -0.54 | 1.79E-02 | 0.36 | 6.00E-01 | 0.06 | 7.75E-01 | 0.96 | 2.75E-02 |
| atcg00560 | (LR) | (ac)T(ph)QSNPNEQSVELNR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| atcg00710 | (LR) | AT(ph)QT(ph)VEDSSR | -0.62 | 4.14E-01 | 0.59 | 6.11E-01 | -0.05 | 6.65E-01 | 1.16 | 1.24E-01 |
| atcg00710 | (LR) | AT(ph)QTVEDSSR | -0.72 | 6.47E-02 | 0.19 | 7.37E-01 | -1.07 | 1.29E-02 | -0.15 | 6.59E-01 |
| atcg00710 | (LR) | ATQT(ph)VEDSSR | -0.84 | 1.94E-01 | 0.28 | 9.17E-01 | -0.64 | 4.36E-01 | 0.48 | 4.80E-01 |

## others - redox.

| | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|
| accession | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G06620 | APPATLT(ph)SPKPPSSSDFSIPTIDLK | -1.92 | 1.00E+00 | | 1.36E-01 | | 4.92E-01 | 1.80 | 1.00E+00 |
| AT3G08710 | GDDDS(ph)VHNVEFSGGNVHLITTK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G08710 | GKGDDDS(ph)VHNVEFSGGNVHLITTK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G08710 | KVTSIIDSVPES(ph)PQRP | -0.71 | 4.99E-01 | -0.12 | 8.42E-01 | 0.50 | 3.84E-01 | 1.09 | 1.37E-01 |
| AT3G08710 | VTSIIDSVPES(ph)PQRP | 0.31 | 4.78E-02 | 0.66 | 1.11E-03 | -0.33 | 8.68E-02 | 0.02 | 9.17E-01 |
| AT3G48890 | DVAT(ph)DDDDAAKE | 0.00 | 9.98E-01 | -0.89 | 1.12E-01 | 0.96 | 6.82E-02 | 0.07 | 7.28E-01 |
| AT3G48890 | KDVAT(ph)DDDDAAKE | 0.34 | 3.26E-01 | -0.57 | 8.63E-02 | 0.61 | 7.26E-03 | -0.30 | 4.11E-01 |
| AT3G48890 | TAS(ph)AEGLSTNTGEEASAITHDETSR | | 1.00E+00 | | 8.63E-02 | | 7.26E-03 | 0.93 | 1.00E+00 |

## others - RNA

(p) processing; (b) binding; (t) transcription

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G02840 | (p) | S(ph)RS(ph)PLPSVQK | -0.24 | 5.51E-01 | -1.34 | 2.05E-02 | 1.47 | 1.39E-03 | 0.37 | 2.55E-01 |
| AT1G20920 | (p) | AWTLEGES(ph)DDEEGHPEEK | | 1.00E+00 | | 1.56E-01 | | 1.25E-01 | | 1.00E+00 |
| AT1G20920 | (p) | EVGNEES(ph)DDDVKR | 0.16 | 5.40E-01 | -0.37 | 2.56E-01 | -0.17 | 5.07E-01 | -0.71 | 3.25E-02 |
| AT1G20920 | (p) | EYGFEEDKS(ph)DS(ph)EDENDVVR | -0.35 | 3.67E-01 | 0.50 | 2.19E-01 | -0.28 | 5.07E-01 | 0.57 | 1.66E-01 |
| AT1G20920 | (p) | SREVGNEES(ph)DDDVKR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G23860 | (p) | S(ph)PDYGYAR | 0.13 | 1.00E+00 | 0.13 | 4.97E-01 | -0.41 | 2.99E-01 | -0.41 | 1.00E+00 |
| AT1G23860 | (p) | S(ph)VT(ph)PPRR | 0.50 | 2.49E-01 | -0.18 | 4.18E-01 | -0.04 | 6.58E-01 | -0.72 | 1.18E-01 |
| AT1G27650 | (p) | RGGS(ph)PGGGREGS(ph)EER | -0.81 | 3.24E-01 | -2.55 | 3.76E-02 | 1.30 | 2.51E-01 | -0.44 | 9.16E-01 |
| AT1G29400 | (b) | NMDLLDS(ph)QLSDDDGRER | -0.02 | 8.23E-01 | -0.89 | 1.68E-01 | 1.37 | 5.70E-02 | 0.51 | 6.01E-01 |
| AT1G29400 | (b) | S(ph)PVFGLS(ph)PTR | 0.14 | 6.14E-01 | 1.09 | 9.37E-03 | -0.85 | 3.12E-02 | 0.11 | 8.16E-01 |
| AT1G30460 | (p) | NEES(ph)ES(ph)EDEDEAPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G30460 | (p) | NEES(ph)ES(ph)EDEDEAPRR | | 1.00E+00 | -0.49 | 1.87E-01 | 1.16 | 5.32E-02 | | 1.00E+00 |
| AT1G32490 | (p) | SGQSDES(ph)DGEVAVR | 1.44 | 7.58E-02 | 0.05 | 9.62E-01 | 0.17 | 7.30E-01 | -1.21 | 4.84E-02 |
| AT1G51510 | (b) | (ac)ANIESEAVDFEPEEDDLM(ox)DEEGTAIDGADVS(ph)PR | -1.29 | 1.92E-02 | | 1.00E+00 | | 1.00E+00 | 0.28 | 8.85E-01 |
| AT1G54080 | (p) | SVVELTNGS(ph)SEDGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G55310 | (p) | GRS(ph)LT(ph)PVR | | 1.00E+00 | 0.90 | 4.15E-02 | -0.26 | 2.41E-01 | | 1.00E+00 |
| AT1G55310 | (p) | S(ph)GDYYS(ph)PPPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G55310 | (p) | S(ph)YT(ph)PEPAR | 0.34 | 6.56E-01 | 0.92 | 2.02E-01 | -0.54 | 4.54E-03 | 0.05 | 7.37E-01 |
| AT1G55310 | (p) | S(ph)YT(ph)PS(ph)PPR | | 1.00E+00 | 1.50 | 2.02E-01 | -1.07 | 4.49E-01 | | 1.00E+00 |
| AT1G55310 | (p) | SYT(ph)PEPAR | 1.36 | 6.16E-02 | 1.61 | 6.18E-03 | -1.25 | 6.47E-02 | -1.00 | 1.67E-02 |
| AT1G55310 | (p) | SYT(ph)PS(ph)PPRGYGR | | 1.00E+00 | -0.58 | 5.47E-03 | 0.43 | 1.02E-01 | | 1.00E+00 |
| AT1G60200 | (p) | ILSGQAAIGSETVQTS(ph)PIENDHK | -0.53 | 9.58E-01 | | 1.00E+00 | | 1.00E+00 | 1.81 | 1.42E-01 |
| AT1G80930 | (b) | DHRAS(ph)DDDEEGEIR | 0.48 | 3.48E-01 | -0.09 | 7.97E-01 | 0.66 | 1.98E-01 | 0.09 | 8.79E-01 |
| AT1G80930 | (b) | ERS(ph)PDVRR | 0.62 | 7.75E-01 | -1.02 | 2.03E-01 | -1.08 | 1.05E-01 | -2.72 | 1.47E-02 |
| AT1G80930 | (b) | ETS(ph)DDEELAR | 0.30 | 6.17E-01 | 0.19 | 6.80E-01 | -0.11 | 7.85E-01 | -0.22 | 7.06E-01 |
| AT1G80930 | (b) | IEVDS(ph)DGDGER | 0.85 | 2.88E-02 | -0.56 | 1.47E-01 | 0.57 | 1.05E-01 | -0.84 | 3.96E-02 |
| AT1G80930 | (b) | IEVDS(ph)DGDGERR | 0.52 | 2.00E-02 | -0.41 | 4.70E-02 | 0.23 | 1.86E-01 | -0.71 | 4.04E-03 |
| AT1G80930 | (b) | RIEVDS(ph)DGDGER | | 1.00E+00 | -0.07 | 9.90E-01 | -0.27 | 7.36E-01 | | 1.00E+00 |
| AT1G80930 | (b) | RKETS(ph)DDEELAR | -0.56 | 6.44E-01 | 0.20 | 6.70E-01 | -0.47 | 5.55E-01 | 0.29 | 7.54E-01 |
| AT1G80930 | (b) | VIADKPS(ph)DEEDDRQR | 0.46 | 1.11E-01 | -0.31 | 8.96E-01 | -0.24 | 7.92E-01 | -1.01 | 5.23E-02 |
| AT1G80930 | (b) | VRVS(ph)DDEDRK | -1.13 | 8.17E-02 | -0.77 | 1.29E-01 | 0.43 | 2.65E-01 | 0.80 | 1.59E-01 |
| AT2G16940 | (b) | EKS(ph)LEIEPK | 0.27 | 1.58E-01 | -1.01 | 4.29E-05 | 1.30 | 7.40E-03 | 0.02 | 8.86E-01 |
| AT2G24590 | (p) | NYS(ph)RS(ph)PPPYR | 0.06 | 9.20E-01 | -0.22 | 5.77E-01 | -0.62 | 4.23E-01 | -0.90 | 2.08E-01 |
| AT2G29210 | (p) | AGLPS(ph)PM(ox)R | 0.27 | 3.25E-01 | 0.53 | 1.00E+00 | -1.08 | 1.00E+00 | -0.82 | 1.98E-01 |
| AT2G29210 | (p) | HHGSQM(ox)S(ph)PVENSEGR | -2.17 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 2.81 | 2.48E-02 |
| AT2G29210 | (p) | HRS(ph)PT(ph)PPAR | | 1.00E+00 | 0.65 | 3.39E-01 | -3.22 | 3.40E-03 | | 1.00E+00 |
| AT2G29210 | (p) | IHS(ph)PFRS(ph)R | | 1.00E+00 | | 1.00E+00 | | 3.40E-03 | | 1.00E+00 |
| AT2G29210 | (p) | LPS(ph)PPPR | 0.33 | 1.93E-01 | | 1.00E+00 | | 1.00E+00 | 0.21 | 6.26E-01 |
| AT2G29210 | (p) | LPS(ph)PPVAQR | 4.50 | 3.18E-03 | 0.58 | 1.63E-01 | -0.30 | 5.20E-01 | -4.22 | 4.18E-05 |
| AT2G29210 | (p) | LPS(ph)PPVAQRLPS(ph)PPPR | -0.48 | 4.13E-01 | -1.01 | 9.93E-02 | 0.66 | 2.62E-01 | 0.12 | 8.43E-01 |
| AT2G29210 | (p) | LPS(ph)PSIEQR | 0.23 | 2.09E-01 | -0.01 | 9.96E-01 | -0.03 | 8.25E-01 | -0.27 | 1.88E-01 |
| AT2G29210 | (p) | RRS(ph)PS(ph)PPAR | -6.62 | 1.00E+00 | | 1.00E+00 | 0.06 | 1.00E+00 | | 1.00E+00 |
| AT2G29210 | (p) | S(ph)LT(ph)PDEERVSLSQGGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G29210 | (p) | SPS(ph)PLYR | 0.46 | 5.97E-02 | | 1.00E+00 | | 1.00E+00 | -0.23 | 1.00E+00 |
| AT2G34750 | (p) | IRPSTS(ph)PES(ph)L | | 1.00E+00 | -0.11 | 1.88E-02 | -0.02 | 3.64E-01 | | 1.00E+00 |
| AT2G37340 | (p) | ARDRS(ph)PVLDDEGS(ph)PK | | 1.00E+00 | -0.43 | 3.06E-01 | -0.13 | 8.01E-01 | | 1.00E+00 |
| AT2G37340 | (p) | DRS(ph)PVLDDEGS(ph)PK | 0.64 | 1.24E-03 | -0.28 | 2.46E-01 | 0.29 | 8.75E-02 | -0.63 | 8.10E-03 |
| AT2G37340 | (p) | EVGS(ph)DRDGGS(ph)PQDNGR | 0.88 | 4.10E-02 | 0.15 | 7.08E-01 | 0.37 | 8.15E-02 | -0.36 | 3.84E-01 |
| AT2G37340 | (p) | EVGSDRDGGS(ph)PQDNGR | 1.27 | 1.86E-04 | -0.35 | 1.74E-01 | -0.05 | 7.53E-01 | -1.66 | 5.43E-06 |
| AT2G37340 | (p) | IIDGS(ph)PPPS(ph)PK | 0.92 | 8.85E-03 | 0.04 | 8.18E-01 | 0.37 | 1.97E-01 | -0.51 | 7.82E-02 |
| AT2G37340 | (p) | MDDS(ph)LS(ph)PR | 0.65 | 3.14E-01 | 0.68 | 4.14E-01 | -0.03 | 8.17E-01 | -0.01 | 9.36E-01 |
| AT2G37340 | (p) | NSVVS(ph)PVVGAGGDSSK | 0.34 | 5.00E-01 | 0.66 | 1.14E-01 | -0.47 | 2.10E-01 | -0.15 | 7.50E-01 |
| AT2G37340 | (p) | NSVVS(ph)PVVGAGGDSSKEDR | 0.64 | 1.59E-01 | -0.60 | 4.11E-01 | 0.05 | 9.29E-01 | -1.18 | 6.00E-02 |
| AT2G37340 | (p) | RM(ox)DDS(ph)LS(ph)PR | 0.73 | 2.31E-02 | 0.44 | 1.91E-01 | -0.72 | 6.61E-02 | -1.02 | 6.57E-03 |
| AT2G37340 | (p) | SPVLDDEGS(ph)PK | 0.46 | 8.33E-02 | | 1.00E+00 | | 1.00E+00 | -0.66 | 5.55E-02 |
| AT2G40650 | (p) | KSVLEDDFEEEEEKEENEGIADGS(ph)EDEM(ox)DQR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G41630 | (t) | EEDLKNLSS(ph)P | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G43410 | (b) | DGS(ph)VDGFTPM(ox)GVDER | -0.08 | 3.19E-01 | -0.22 | 4.41E-01 | -0.53 | 1.99E-01 | -0.66 | 2.60E-02 |
| AT3G01540 | (p) | APPPS(ph)STGSPPR | | 1.00E+00 | 0.47 | 2.39E-01 | | 1.00E+00 | 0.52 | 2.25E-01 |
| AT3G01540 | (p) | APPPSS(ph)TGSPPR | | 1.00E+00 | -0.39 | 1.00E+00 | 0.66 | 7.56E-02 | | 1.00E+00 |
| AT3G01540 | (p) | APPPSST(ph)GSPPR | 1.15 | 1.00E+00 | | 1.00E+00 | -0.48 | 7.56E-02 | | 1.00E+00 |
| AT3G01540 | (p) | VPLPSSAPASELS(ph)PEAYSR | 0.60 | 5.11E-02 | 0.24 | 4.44E-01 | 0.33 | 3.15E-01 | -0.03 | 7.72E-01 |
| AT3G08620 | (b) | (ac)SGLYNYNNFS(ph)PSR | -0.82 | 4.44E-02 | 1.00 | 1.73E-02 | -1.89 | 1.73E-03 | -0.08 | 8.15E-01 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT3G13300 | (p) | S(ph)ESANKLSFPSAEATSQAIVPPNGEPK | -0.52 | 5.62E-01 | | 1.00E+00 | | 1.00E+00 | 1.20 | 1.01E-01 |
| AT3G13300 | (p) | SKDSNVTPDDDVS(ph)GM(ox)RSPSAFFK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.01E-01 |
| AT3G13300 | (p) | TLSYPTPPLNLQS(ph)PR | -0.32 | 3.82E-01 | -0.88 | 6.16E-02 | 0.68 | 2.40E-01 | 0.13 | 8.64E-01 |
| AT3G13300 | (p) | TSGLPSQTSGAGSAYATLPQLPLS(ph)PR | 0.00 | 4.30E-01 | -2.56 | 4.24E-02 | 2.71 | 1.00E+00 | 0.14 | 6.53E-01 |
| AT3G13570 | (p) | S(ph)YTPEQAR | 1.03 | 1.09E-02 | 0.79 | 3.36E-02 | -0.40 | 5.57E-02 | -0.64 | 5.69E-02 |
| AT3G14100 | (b) | GATSGDDKLS(ph)SDGK | 0.53 | 1.18E-01 | -1.12 | 6.66E-02 | 0.97 | 1.00E+00 | -0.69 | 3.02E-01 |
| AT3G26560 | (p) | YSVDM(ox)S(ph)PVK | 0.10 | 7.44E-01 | -0.01 | 9.74E-01 | -0.28 | 2.80E-01 | -0.38 | 2.24E-01 |
| AT3G27700 | (b) | (ac)M(ox)ELSVS(ph)SPK | | 1.00E+00 | 0.60 | 4.10E-01 | -1.06 | 1.77E-01 | | 1.00E+00 |
| AT3G50670 | (p) | ELS(ph)HEQPR | | 1.00E+00 | 1.15 | 3.04E-02 | -0.09 | 3.26E-01 | | 1.00E+00 |
| AT3G50670 | (p) | TSQS(ph)EEPSRPR | 0.63 | 7.58E-02 | 0.27 | 3.63E-01 | -0.39 | 2.72E-01 | -0.74 | 3.76E-02 |
| AT3G53500 | (b) | GRDQS(ph)LS(ph)PDRK | 0.20 | 7.86E-01 | -1.21 | 1.71E-03 | 0.85 | 1.53E-02 | -0.56 | 2.52E-01 |
| AT3G53500 | (b) | KVIDAS(ph)PK | | 1.00E+00 | -0.10 | 4.50E-01 | | 1.00E+00 | | 1.00E+00 |
| AT3G53500 | (b) | S(ph)PIDDEAELSRPS(ph)PK | 0.03 | 3.86E-01 | 0.80 | 2.11E-01 | -0.70 | 1.30E-01 | 0.07 | 2.31E-01 |
| AT3G53500 | (b) | VIDAS(ph)PK | | 1.00E+00 | | 1.97E-02 | | 9.67E-02 | | 1.00E+00 |
| AT3G55460 | (p) | GRS(ph)PPPPPPR | 0.09 | 3.70E-01 | -0.21 | 4.39E-01 | 0.43 | 1.09E-01 | 0.13 | 9.05E-01 |
| AT3G55460 | (p) | RYS(ph)PPYYS(ph)PPR | 0.06 | 4.27E-01 | 0.56 | 2.79E-01 | -0.30 | 6.25E-01 | 0.20 | 8.91E-01 |
| AT3G55460 | (p) | S(ph)PPPPPPR | 0.49 | 3.93E-01 | 0.75 | 2.81E-01 | -0.15 | 9.30E-01 | 0.12 | 8.37E-01 |
| AT3G55460 | (p) | S(ph)VEVS(ph)PR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.58 | 1.22E-01 |
| AT3G55460 | (p) | S(ph)YS(ph)PGYEGAAAAAPDR | 0.16 | 9.02E-01 | 0.80 | 6.38E-02 | -0.69 | 2.20E-01 | -0.04 | 6.80E-01 |
| AT3G55460 | (p) | S(ph)YS(ph)PGYEGAAAAAPDRDR | 0.14 | 4.63E-01 | -0.35 | 6.53E-01 | 0.23 | 7.53E-01 | -0.25 | 3.80E-01 |
| AT3G61860 | (p) | QRS(ph)PGYDR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G61860 | (p) | RPS(ph)PDYGR | 0.53 | 1.19E-01 | 0.05 | 9.75E-01 | -0.05 | 7.92E-01 | -0.53 | 1.83E-01 |
| AT3G61860 | (p) | RS(ph)LS(ph)PVYR | -0.03 | 9.58E-01 | 0.56 | 7.34E-01 | -0.44 | 8.69E-01 | 0.16 | 9.13E-01 |
| AT4G12610 | (t) | SSGGDEEEGNVS(ph)DRGDEDEEEEASR | 0.94 | 1.33E-01 | -0.62 | 5.70E-02 | 0.82 | 1.00E+00 | -0.75 | 2.84E-01 |
| AT4G12610 | (t) | SSGGDEEEGNVS(ph)DRGDEDEEEEASRK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.51 | 4.42E-01 |
| AT4G17720 | (b) | LSES(ph)PEAK | 0.10 | 3.89E-01 | 1.48 | 1.00E+00 | -0.73 | 1.00E+00 | 0.65 | 2.82E-01 |
| AT4G17720 | (b) | VHLSES(ph)PK | 0.30 | 6.79E-02 | 1.16 | 1.85E-02 | -0.24 | 8.04E-02 | 0.62 | 5.42E-02 |
| AT4G20910 | (p) | SSS(ph)PNVFAAPPILQK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G25500 | (p) | DSDGGYDGAES(ph)PM(ox)QK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G25500 | (p) | GAS(ph)PVAAYR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G25500 | (p) | NGVGEVES(ph)PIER | 1.06 | 1.37E-03 | 0.12 | 5.02E-01 | -0.07 | 7.36E-01 | -1.01 | 1.92E-03 |
| AT4G25500 | (p) | NGVGEVES(ph)PIERR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G25500 | (p) | RGS(ph)PEYGRDR | | 1.00E+00 | -1.56 | 1.07E-02 | 0.06 | 2.23E-01 | | 1.00E+00 |
| AT4G25500 | (p) | SRS(ph)PRS(ph)PPADE | 0.89 | 3.41E-02 | | 1.00E+00 | | 1.00E+00 | -0.55 | 6.20E-01 |
| AT4G28990 | (b) | DYS(ph)PPLAR | 0.37 | 9.62E-01 | 0.42 | 6.30E-01 | -0.30 | 3.94E-01 | -0.25 | 7.64E-01 |
| AT4G28990 | (b) | YAPANS(ph)PPLPR | 1.39 | 2.36E-02 | 0.21 | 3.60E-01 | 0.28 | 3.34E-01 | -0.90 | 2.33E-02 |
| AT4G31200 | (p) | EIGEVNPSEGPMGSESQDDYDNYERDS(ph)PQRK | | 3.34E-01 | | 1.00E+00 | | 1.00E+00 | | 3.87E-01 |
| AT4G31580 | (p) | ARS(ph)PPPPR | -2.32 | 4.75E-01 | -1.44 | 4.32E-01 | -0.49 | 6.82E-01 | 0.38 | 6.63E-01 |
| AT4G31580 | (p) | RRS(ph)PS(ph)PPPAR | -0.93 | 1.71E-02 | -0.16 | 4.61E-01 | 0.12 | 5.30E-01 | 0.89 | 1.98E-02 |
| AT4G31580 | (p) | SYS(ph)RS(ph)PPPYR | -0.50 | 1.45E-02 | | 1.00E+00 | | 1.00E+00 | 0.23 | 6.37E-01 |
| AT4G35785 | (b) | S(ph)LPRPVS(ph)PSR | 0.13 | 6.05E-01 | -0.07 | 1.00E+00 | -0.36 | 6.46E-01 | -0.56 | 3.50E-01 |
| AT4G35785 | (b) | T(ph)PTPGHYLGLK | 0.68 | 2.60E-01 | | 1.00E+00 | | 1.00E+00 | -0.46 | 5.32E-01 |
| AT4G35800 | (t) | YS(ph)PSIAYS(ph)PSNAR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G04430 | (b) | (ac)M(ox)ESTESYAAGS(ph)PEELAKR | 0.50 | 1.36E-01 | 0.59 | 1.29E-01 | -0.52 | 1.00E-01 | -0.43 | 1.34E-01 |
| AT5G04430 | (b) | (ac)MESTESYAAGS(ph)PEELAK | 0.20 | 4.65E-01 | 1.00 | 2.08E-02 | -0.47 | 2.23E-01 | 0.32 | 4.57E-01 |
| AT5G06160 | (p) | EGEEANTELES(ph)DDEDGLIYNPLK | 0.09 | 8.99E-01 | -0.63 | 2.34E-01 | 1.40 | 4.48E-02 | 0.69 | 2.04E-01 |
| AT5G15270 | (b) | SEDS(ph)PEGEKQVTAK | 0.72 | 3.23E-01 | -0.67 | 3.02E-01 | 0.28 | 1.47E-02 | -1.11 | 2.24E-01 |
| AT5G15270 | (b) | VVADDARS(ph)EDS(ph)PEGEK | 2.38 | 3.79E-03 | 0.85 | 3.69E-01 | 0.13 | 6.14E-01 | -1.40 | 1.00E+00 |
| AT5G16260 | (b) | ASS(ph)PPEGEDEFTDDDGTK | 0.32 | 3.54E-01 | 0.24 | 1.04E-01 | 0.07 | 4.58E-01 | 0.00 | 9.86E-01 |
| AT5G23080 | (p) | SSS(ph)S(ph)RYSSDEEQKESR | 1.36 | 1.00E+00 | -1.26 | 8.95E-02 | 1.36 | 4.48E-02 | -1.26 | 4.01E-02 |
| AT5G37370 | (p) | NGGDEVQQRS(ph)PR | | 1.00E+00 | -1.49 | 3.39E-04 | 1.29 | 7.43E-02 | | 1.00E+00 |
| AT5G38600 | (p) | NSLES(ph)GNGSPEANSLVGNDENVK | | 1.00E+00 | 0.94 | 2.27E-01 | -1.12 | 1.48E-01 | | 1.00E+00 |
| AT5G44200 | (p) | FRESGDS(ph)DDDGEDDR | 0.98 | 3.39E-01 | 0.51 | 7.25E-01 | -0.18 | 8.02E-01 | -0.66 | 3.86E-01 |
| AT5G44200 | (p) | FRESGDS(ph)DDDGEDDRK | -0.38 | 1.00E-01 | | 1.00E+00 | | 1.00E+00 | 0.92 | 1.05E-01 |
| AT5G51300 | (p) | TLS(ph)GNDKDQS(ph)GGEEETTSR | 0.06 | 4.25E-01 | -0.46 | 5.21E-01 | 0.17 | 9.92E-01 | -0.36 | 2.33E-01 |
| AT5G52040 | (p) | ARLS(ph)PDYK | 0.39 | 2.49E-01 | -0.76 | 5.00E-03 | 0.28 | 6.32E-02 | -0.87 | 1.87E-02 |
| AT5G52040 | (p) | ARLS(ph)PDYKR | | 1.00E+00 | | 5.00E-03 | | 6.32E-02 | 0.73 | 1.00E+00 |
| AT5G52040 | (p) | DDDSRGNGYS(ph)PER | 0.52 | 1.63E-01 | -0.52 | 5.00E-02 | 0.23 | 3.24E-01 | -0.81 | 3.96E-02 |
| AT5G52040 | (p) | DDDSRGNGYS(ph)PERR | 1.07 | 3.06E-02 | -0.11 | 9.77E-01 | 0.00 | 9.91E-01 | -1.18 | 1.92E-01 |
| AT5G52040 | (p) | ERGS(ph)PDYGR | -0.76 | 2.73E-03 | 0.16 | 2.79E-01 | -0.50 | 2.21E-02 | 0.42 | 3.88E-02 |
| AT5G52040 | (p) | ERT(ph)SPDYGR | 0.02 | 7.49E-01 | -1.08 | 8.24E-02 | 0.74 | 2.43E-01 | -0.36 | 6.26E-01 |
| AT5G52040 | (p) | ERVAS(ph)PENGAVR | 0.75 | 1.64E-01 | -0.97 | 1.54E-01 | 0.81 | 1.95E-01 | -0.91 | 1.26E-01 |
| AT5G52040 | (p) | GES(ph)RS(ph)PPPYEK | 0.29 | 6.75E-01 | -0.83 | 8.11E-01 | 0.83 | 4.39E-01 | -0.29 | 7.84E-01 |
| AT5G52040 | (p) | GNGYS(ph)PER | | 5.06E-02 | | 4.35E-01 | | 1.41E-01 | 1.40 | 1.81E-01 |
| AT5G52040 | (p) | GYDGADS(ph)PIRES(ph)PSRS(ph)PPAEE | 1.13 | 1.52E-01 | -0.23 | 9.04E-01 | 0.26 | 8.07E-01 | -1.09 | 1.83E-01 |
| AT5G52040 | (p) | LS(ph)PDYK | | 1.00E+00 | 0.37 | 2.99E-01 | -0.67 | 2.63E-02 | | 1.00E+00 |
| AT5G52040 | (p) | RES(ph)RS(ph)PPPYEK | 0.14 | 7.50E-01 | -0.43 | 3.50E-01 | 0.11 | 6.65E-01 | -0.46 | 4.37E-01 |
| AT5G52040 | (p) | S(ph)PPPYEKR | 2.04 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -2.88 | 1.00E+00 |
| AT5G52040 | (p) | SKSS(ph)PENGQVES(ph)PGQIMEVEAGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G52040 | (p) | VAS(ph)PENGAVR | 1.33 | 3.08E-03 | -0.01 | 8.10E-01 | 0.18 | 4.93E-01 | -1.15 | 3.33E-02 |
| AT5G62190 | (p) | LKLS(ph)DS(ph)DEEESEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.48 | 1.32E-01 |
| AT5G62190 | (p) | LSDS(ph)DEEESEKKK | -2.82 | 1.03E-02 | -0.28 | 8.66E-01 | -0.38 | 4.17E-01 | 2.16 | 4.73E-02 |

## others

(Co) Co-factor and vitamine metabolism; (mi) micro RNA; (sec) secondary metabolism; (TCA) TCA/org transformation; (na) not assigned

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G01320 | (na) | SPS(ph)YKEVALAPPGSIAK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G02870 | (na) | EFEPIDS(ph)GS(ph)ELEEDDLK | | 1.00E+00 | | 2.05E-02 | | 1.39E-03 | | 1.00E+00 |
| AT1G02870 | (na) | EFEPIDS(ph)GS(ph)ELEEDDLKTALGK | | 1.00E+00 | | 2.05E-02 | | 1.39E-03 | | 1.00E+00 |
| AT1G03350 | (na) | SGGS(ph)PNRAELR | -0.38 | 1.24E-02 | -0.17 | 6.62E-01 | -0.02 | 9.49E-01 | 0.19 | 2.23E-01 |
| AT1G03910 | (na) | LEQLS(ph)EGEDDVEVNPGLTR | 0.19 | 3.55E-01 | -0.38 | 5.04E-02 | 0.99 | 5.37E-05 | 0.42 | 5.08E-02 |
| AT1G04080 | (na) | SQVDGST(ph)EQSPK | -1.44 | 1.00E+00 | 1.49 | 1.17E-01 | -1.34 | 1.29E-01 | 1.59 | 1.00E+00 |
| AT1G04080 | (na) | SQVDGSTEQS(ph)PK | -0.05 | 1.00E+00 | -0.61 | 5.18E-02 | 1.17 | 1.00E+00 | 0.61 | 5.16E-02 |
| AT1G04080 | (na) | SQVDGSTEQS(ph)PKLESASSTEPEELK | | 1.00E+00 | | 5.18E-02 | | 1.00E+00 | | 5.16E-02 |
| AT1G04080 | (na) | SQVDGSTEQS(ph)PKLESASSTEPEELKK | -0.01 | 4.47E-01 | | 1.00E+00 | | 1.00E+00 | 0.39 | 4.92E-02 |
| AT1G04510 | (na) | IFGLPDDDNTEDS(ph)AQDS | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G05500 | (na) | VLKNDTTDEENAS(ph)SR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G06190 | (na) | AELVELLGSDSS(ph) | | 1.00E+00 | 0.24 | 1.38E-01 | | 1.00E+00 | | 1.00E+00 |
| AT1G06190 | (na) | KAELVELLGSDS(ph)S | 0.16 | 7.82E-01 | -1.00 | 7.34E-02 | 0.74 | 4.64E-02 | -0.42 | 5.06E-01 |
| AT1G06890 | (na) | ESEKDPLIAAENGSGVLS(ph)DGGGGVQQK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G07090 | (na) | SDPVKGDDPGPSFVSS(ph)PPATPSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 9.04E-02 |
| AT1G08800 | (na) | GIEFLS(ph)PR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G10990 | (na) | TNS(ph)PPPSALPK | -0.35 | 1.47E-01 | 0.06 | 9.85E-01 | -0.51 | 2.56E-01 | -0.10 | 8.05E-01 |
| AT1G11700 | (na) | QLAS(ph)SAPVNVPDWSK | | 8.97E-02 | -0.93 | 1.00E+00 | -0.12 | 1.00E+00 | | 1.00E+00 |
| AT1G12530 | (na) | TDFNTS(ph)PASK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G13030 | (na) | LDTTEES(ph)PDER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G13030 | (na) | LDTTEES(ph)PDERENTAVVSNVVK | -0.68 | 3.28E-01 | -0.15 | 7.23E-01 | -0.17 | 8.16E-01 | 0.36 | 6.29E-01 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G13380 | (na) | S(ph)SSNIGM(ox)AGYA | -0.35 | 2.40E-01 | 1.21 | 6.79E-02 | -1.01 | 1.51E-01 | 0.55 | 1.00E+00 |
| AT1G14170 | (na) | VHDM(ox)VVADADQDDGT(ph)DDDNDLGEK | 0.55 | 1.83E-01 | 0.56 | 9.70E-04 | -0.19 | 1.55E-01 | -0.18 | 4.68E-01 |
| AT1G14170 | (na) | VHDMVVADADQDDGT(ph)DDDNDLGEK | 0.57 | 5.83E-02 | 0.12 | 7.52E-01 | 0.59 | 4.46E-02 | 0.13 | 6.32E-01 |
| AT1G15280 | (na) | (ac)ATSEAEYES(ph)DPEELNR | 0.00 | 9.54E-01 | 0.98 | 5.29E-03 | -0.89 | 8.67E-03 | 0.10 | 8.88E-01 |
| AT1G15280 | (na) | AVVDS(ph)DLS(ph)DEEVGTVK | 0.00 | 8.60E-01 | 0.44 | 1.39E-01 | 0.14 | 5.56E-01 | 0.57 | 9.55E-02 |
| AT1G15280 | (na) | RREAS(ph)DDDS(ph)DDDDAVR | 0.46 | 1.17E-01 | 0.47 | 7.58E-01 | 0.12 | 6.42E-01 | 0.13 | 2.64E-01 |
| AT1G15280 | (na) | YDNDEDGEDS(ph)YEDDEEESGGGIDNDK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G15400 | (na) | QGS(ph)SGIVFDDR | 0.34 | 3.11E-01 | 0.54 | 7.48E-02 | -0.23 | 4.63E-01 | -0.03 | 9.36E-01 |
| AT1G15400 | (na) | RQGS(ph)SGIVFDDR | | 1.00E+00 | 1.30 | 4.54E-02 | | 1.00E+00 | | 1.00E+00 |
| AT1G15400 | (na) | STIS(ph)FR | -0.03 | 9.30E-01 | 0.23 | 5.85E-01 | 0.19 | 6.90E-01 | 0.46 | 3.87E-01 |
| AT1G15400 | (na) | TTGRVS(ph)PAVDPPS(ph)PR | 0.01 | 9.56E-01 | 0.59 | 3.63E-01 | 0.04 | 8.24E-01 | 0.62 | 2.94E-01 |
| AT1G15400 | (na) | VS(ph)PAVDPPS(ph)PR | 0.78 | 7.47E-02 | 0.85 | 4.25E-02 | 0.08 | 8.84E-01 | 0.15 | 5.92E-01 |
| AT1G15400 | (na) | VSPAVDPPS(ph)PR | 0.26 | 4.76E-01 | 0.51 | 2.03E-01 | -0.18 | 7.07E-01 | 0.07 | 7.83E-01 |
| AT1G15440 | (na) | GLES(ph)DEEGDDDDEEYMHR | 0.60 | 4.48E-01 | -0.06 | 4.78E-01 | 0.61 | 1.85E-01 | -0.05 | 9.45E-01 |
| AT1G15440 | (na) | KM(ox)TEAGPIDLIDDDNS(ph)DEEGGIDK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G15440 | (na) | M(ox)TEAGPIDLIDDDNS(ph)DEEGGIDK | -0.11 | 5.55E-01 | 0.54 | 1.11E-02 | -0.17 | 3.41E-01 | 0.48 | 2.03E-02 |
| AT1G15440 | (na) | M(ox)TEAGPIDLIDDDNS(ph)DEEGGIDKQSR | -1.00 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.60 | 3.41E-02 |
| AT1G15630 | (na) | (ac)M(ox)ES(ph)PLLTK | | 1.00E+00 | | 1.00E+00 | -0.02 | 4.01E-01 | | 1.00E+00 |
| AT1G15950 | (sec) | PVDVAS(ph)PAGK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G16170 | (na) | VLDGLVS(ph)SPSR | | 1.00E+00 | 1.10 | 5.14E-03 | -1.28 | 1.66E-02 | | 1.00E+00 |
| AT1G16180 | (na) | AGSSTTLLSPPDS(ph)PR | 0.06 | 1.00E+00 | 0.23 | 2.74E-01 | 0.26 | 1.00E+00 | 0.43 | 1.85E-01 |
| AT1G16520 | (na) | IISTSVS(ph)PR | 0.67 | 5.65E-02 | -0.44 | 1.00E+00 | 0.17 | 2.29E-01 | -0.95 | 2.37E-02 |
| AT1G16860 | (na) | KVS(ph)GPLDSSGLM(ox)K | | 1.00E+00 | 1.25 | 7.87E-03 | -2.62 | 1.00E+00 | | 1.00E+00 |
| AT1G16860 | (na) | QNS(ph)GSIPILPATGLITSGPITSGPLNSSGAPR | -1.36 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 2.33 | 9.58E-02 |
| AT1G16860 | (na) | S(ph)GPIPGAPSR | 0.19 | 6.12E-01 | 0.42 | 2.94E-01 | -0.35 | 4.00E-01 | -0.12 | 7.90E-01 |
| AT1G17210 | (na) | ADS(ph)VEGTVVDR | | 1.00E+00 | | 7.74E-02 | | 1.80E-01 | | 1.00E+00 |
| AT1G18740 | (na) | SLS(ph)WSVSR | | 1.00E+00 | 0.59 | 4.56E-02 | | 1.00E+00 | | 1.00E+00 |
| AT1G20770 | (na) | DFSTDTDSEIGSPLS(ph)PQLR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G20970 | (na) | SELES(ph)TDDGPEEVVEIPK | 0.25 | 6.88E-01 | 1.76 | 1.00E+00 | -0.09 | 3.84E-01 | 1.41 | 1.19E-01 |
| AT1G21170 | (na) | VVLTSLQS(ph)FPR | 1.67 | 1.88E-02 | 0.17 | 9.51E-01 | 0.51 | 3.10E-01 | -0.99 | 8.51E-02 |
| AT1G22060 | (na) | SVVSGDLSGLAQS(ph)PQKEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G24190 | (na) | VEREEGELS(ph)PNGDFEEDNFAVYAK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G24267 | (na) | TLSLPPAS(ph)PR | 2.15 | 1.73E-03 | | 1.00E+00 | | 1.00E+00 | -1.98 | 2.21E-03 |
| AT1G24300 | (na) | NNS(ph)LLSGIIDGGR | 0.38 | 3.02E-01 | -2.35 | 2.36E-03 | 2.76 | 1.58E-03 | 0.03 | 5.00E-01 |
| AT1G24560 | (na) | (ac)ANGADEDAVLS(ph)DVESDEPAPVVLK | 0.04 | 9.82E-01 | | 1.00E+00 | | 1.00E+00 | 0.40 | 4.45E-02 |
| AT1G26300 | (na) | IGNS(ph)DVEDEK | 0.98 | 1.00E+00 | 0.46 | 1.00E+00 | 0.74 | 1.00E+00 | 0.21 | 1.00E+00 |
| AT1G27100 | (na) | FASLGLSTS(ph)PR | | 1.00E+00 | 0.10 | 4.72E-01 | | 1.00E+00 | | 1.00E+00 |
| AT1G28280 | (na) | LLPLFPVT(ph)SPR | | 1.00E+00 | -0.36 | 7.03E-02 | 0.79 | 1.08E-01 | | 1.00E+00 |
| AT1G28380 | (na) | S(ph)GVFSM(ox)ISTR | | 1.00E+00 | 2.24 | 7.03E-02 | | 1.08E-01 | | 1.00E+00 |
| AT1G31810 | (na) | SLLS(ph)R | 0.07 | 7.40E-01 | 0.20 | 8.81E-01 | -0.44 | 5.70E-01 | -0.32 | 4.13E-01 |
| AT1G31870 | (na) | QVDPEEDENEDDS(ph)AEETPLVDEDIEVKR | | 1.00E+00 | | 2.93E-01 | | 1.33E-01 | | 1.00E+00 |
| AT1G31870 | (na) | RDS(ph)SPPQISK | 0.18 | 4.45E-02 | | 1.00E+00 | | 1.00E+00 | -0.14 | 2.66E-01 |
| AT1G31870 | (na) | SFGSNADLS(ph)PPGR | 0.33 | 7.09E-02 | -0.83 | 1.25E-02 | 0.76 | 8.02E-04 | -0.40 | 4.18E-02 |
| AT1G31870 | (na) | SNDLS(ph)PPR | | 1.00E+00 | -0.91 | 3.08E-01 | 1.66 | 1.00E+00 | | 1.00E+00 |
| AT1G31870 | (na) | SSNFDSS(ph)PPR | | 1.00E+00 | | 3.08E-01 | | 1.00E+00 | | 1.00E+00 |
| AT1G31870 | (na) | YHS(ph)PS(ph)PEPARR | 0.58 | 2.57E-01 | | 1.00E+00 | | 1.00E+00 | 0.47 | 4.70E-01 |
| AT1G31870 | (na) | YLS(ph)EDLS(ph)PPR | -0.72 | 5.08E-01 | 1.83 | 2.43E-02 | -0.45 | 5.57E-01 | 2.09 | 1.44E-01 |
| AT1G33050 | (na) | S(ph)TDDLSGFR | | 7.58E-02 | | 1.00E+00 | | 1.00E+00 | | 4.84E-02 |
| AT1G34020 | (na) | LGAAS(ph)DS(ph)DDNEDKA | 0.08 | 8.63E-01 | -0.46 | 1.83E-01 | 0.16 | 3.24E-01 | -0.38 | 4.79E-01 |
| AT1G34320 | (na) | S(ph)QEFETVAK | 0.62 | 3.00E-01 | 1.07 | 9.46E-02 | -0.45 | 4.13E-01 | 0.00 | 9.56E-01 |
| AT1G35510 | (na) | INIPSPS(ph)PPS(ph)SPR | 1.79 | 1.00E+00 | 0.29 | 4.93E-01 | -0.24 | 9.91E-02 | -1.74 | 7.53E-02 |
| AT1G44910 | (na) | ANLS(ph)PAGDKANVEEPM(ox)VYATK | -0.36 | 2.24E-01 | 0.02 | 9.69E-01 | -0.25 | 3.16E-01 | 0.12 | 7.81E-01 |
| AT1G44910 | (na) | KHANS(ph)PES(ph)ESENR | -0.54 | 4.59E-01 | -0.16 | 9.46E-01 | 0.40 | 8.51E-01 | 0.79 | 4.96E-01 |
| AT1G45688 | (na) | TDSEVTSLAASSPARS(ph)PR | | 1.00E+00 | 0.05 | 8.30E-01 | -0.12 | 7.11E-01 | | 1.00E+00 |
| AT1G47330 | (na) | DLDEQEQS(ph)PETSENGIER | | 1.00E+00 | 0.74 | 1.79E-03 | -0.27 | 5.38E-02 | | 1.00E+00 |
| AT1G47900 | (na) | VSGYES(ph)DSKLQEIEELR | 0.47 | 4.31E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G47970 | (na) | AEDEEDAS(ph)DFEPEENGVEEDIDEGEDDENDNSGGAGK | -0.31 | 3.72E-01 | 0.24 | 3.68E-01 | 0.26 | 5.46E-01 | 0.81 | 9.09E-03 |
| AT1G47970 | (na) | APEEDEEDS(ph)GDEDDDRPPK | 0.46 | 7.33E-01 | 0.72 | 2.30E-01 | -0.17 | 5.94E-01 | 0.09 | 7.56E-01 |
| AT1G47970 | (na) | APEEDEEDS(ph)GDEDDDRPPKR | -0.02 | 2.77E-01 | -0.16 | 6.71E-01 | -0.02 | 8.20E-01 | -0.16 | 1.99E-01 |
| AT1G47970 | (na) | EEPEIQVLS(ph)DDDS(ph)DEEQVK | 0.71 | 5.45E-01 | 0.28 | 7.15E-01 | 0.66 | 4.51E-01 | 0.22 | 6.89E-01 |
| AT1G47970 | (na) | NFKEEPEIQVLS(ph)DDDS(ph)DEEQVK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G47970 | (na) | RAPEEDEEDS(ph)GDEDDDRPPK | | 1.00E+00 | -0.16 | 4.36E-01 | | 1.00E+00 | -0.54 | 6.79E-01 |
| AT1G47970 | (na) | TGVNDDDDKNFKEEPEIQVLS(ph)DDDS(ph)DEEQVK | -0.45 | 4.66E-01 | -0.03 | 4.49E-01 | 0.72 | 4.89E-02 | 1.13 | 1.08E-01 |
| AT1G50120 | (na) | TFGSSLS(ph)LNSGPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G52200 | (na) | GRVTTPSEEDSNNGLPVQQPGT(ph)PNQR | 0.59 | 5.21E-02 | 0.42 | 3.81E-01 | -0.77 | 1.01E-01 | -0.94 | 1.24E-02 |
| AT1G52200 | (na) | VTTPSEEDSNNGLPVQQPGT(ph)PNQR | 0.11 | 7.71E-01 | 0.86 | 1.25E-04 | -0.37 | 3.73E-02 | 0.38 | 3.85E-02 |
| AT1G52220 | (na) | AS(ph)GESSDSSTDLDVVSTIQNVWDK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G52320 | (na) | VSS(ph)PPRVPNPAIQK | | 1.00E+00 | 1.16 | 9.22E-02 | -0.57 | 3.84E-01 | | 1.00E+00 |
| AT1G52780 | (na) | VM(ox)S(ph)ESEM(ox)VSGAR | 0.09 | 8.27E-01 | -0.06 | 7.90E-01 | -0.21 | 5.85E-01 | -0.36 | 3.57E-01 |
| AT1G53590 | (na) | EEFLIGSIEEESQSQS(ph)PR | -0.61 | 4.29E-01 | -0.43 | 3.36E-01 | 0.72 | 1.41E-02 | 0.89 | 2.13E-01 |
| AT1G53590 | (na) | KEEFLIGSIEEESQSQS(ph)PR | -1.89 | 2.19E-01 | | 1.00E+00 | | 1.00E+00 | 0.56 | 9.62E-01 |
| AT1G56230 | (na) | SLS(ph)EISEVDAVR | 1.05 | 7.53E-02 | 0.36 | 7.41E-01 | 0.10 | 5.57E-01 | -0.59 | 2.59E-01 |
| AT1G57680 | (na) | SGALLLAPNSSQTDEGLS(ph)LR | -0.50 | 5.96E-02 | 0.01 | 3.99E-01 | 0.09 | 3.89E-01 | 0.61 | 1.01E-01 |
| AT1G59710 | (na) | QESTDSLAVGS(ph)PPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G59710 | (na) | QESTDSLAVGS(ph)PPKSEGR | -0.52 | 9.02E-01 | 0.65 | 5.41E-01 | -1.91 | 1.12E-01 | -0.74 | 3.19E-01 |
| AT1G59900 | (TCA) | YHGHS(ph)M(ox)SDPGSTYR | | 3.19E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G65010 | (na) | SEVS(ph)PERETELDSVEEEVDSK | -0.09 | 8.06E-01 | -0.56 | 1.60E-01 | 0.40 | 1.43E-01 | -0.07 | 6.77E-01 |
| AT1G65320 | (na) | SIGFNPTS(ph)PT(ph)RLSIGR | | 1.00E+00 | -1.63 | 1.22E-05 | 2.02 | 1.95E-03 | | 1.00E+00 |
| AT1G65320 | (na) | SIGFNPTS(ph)PTR | 0.45 | 9.67E-02 | 1.55 | 4.11E-03 | -0.89 | 4.25E-02 | 0.21 | 1.19E-01 |
| AT1G65370 | (na) | TVWLQQWIM(ox)AAT(ph)KAR | | 9.67E-02 | | 1.00E+00 | | 1.00E+00 | | 1.19E-01 |
| AT1G66680 | (na) | AAAAVTTTTTDSLAS(ph)DDDR | 0.31 | 2.31E-01 | 0.63 | 2.62E-01 | -0.26 | 5.79E-01 | 0.07 | 1.13E-01 |
| AT1G67230 | (na) | ADS(ph)DGEDDES(ph)DAEHPGK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G67230 | (na) | ALYGESINLYEPNDS(ph)TENVDDSTK | -0.06 | 9.69E-01 | -0.27 | 1.47E-01 | 0.36 | 5.09E-04 | 0.15 | 6.55E-01 |
| AT1G67230 | (na) | AQEVAADS(ph)LSNLDVDGQSR | 0.29 | 1.49E-01 | 0.18 | 3.81E-01 | -0.36 | 1.02E-01 | -0.46 | 2.93E-02 |
| AT1G67230 | (na) | AQEVAADSLS(ph)NLDVDGQSR | 0.12 | 4.23E-01 | 0.22 | 3.32E-01 | -0.16 | 5.82E-01 | -0.06 | 7.50E-01 |
| AT1G67230 | (na) | DIS(ph)PTAAGLGLPVTGGK | 0.55 | 1.28E-02 | 0.63 | 8.44E-03 | 0.08 | 6.76E-01 | 0.16 | 4.95E-01 |
| AT1G67230 | (na) | EVEVTNVNS(ph)DGDQSDINSK | 0.71 | 2.01E-01 | 1.25 | 1.98E-02 | -0.82 | 9.80E-02 | -0.28 | 6.23E-01 |
| AT1G67230 | (na) | S(ph)VKDVVDDAK | -0.48 | 2.67E-01 | 0.49 | 2.27E-01 | -0.86 | 6.98E-02 | 0.11 | 8.09E-01 |
| AT1G68790 | (na) | ITESEQAAGDS(ph)DEGVDSITTGGR | 0.56 | 2.21E-01 | 0.70 | 2.18E-01 | -0.53 | 3.26E-01 | -0.39 | 3.31E-01 |
| AT1G69070 | (na) | DDFDSGLLS(ph)DEDLQDDDLEASASK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.50 | 1.88E-01 |
| AT1G69070 | (na) | M(ox)QETEELS(ph)DGDEEIGGEESTKR | | 1.00E+00 | 0.42 | 1.17E-01 | -0.74 | 1.86E-01 | | 1.00E+00 |
| AT1G70180 | (na) | DIS(ph)PPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G70180 | (na) | DRS(ph)PPRSDGR | 1.13 | 1.00E-01 | -0.52 | 7.41E-01 | -0.62 | 6.79E-01 | -2.27 | 3.61E-02 |
| AT1G70770 | (na) | MTAIDS(ph)DDDGVVR | 1.43 | 4.66E-02 | -0.03 | 9.78E-01 | 0.77 | 4.26E-01 | -0.68 | 1.62E-01 |
| AT1G70770 | (na) | SNGYGDDGYDFDGS(ph)DDEIATLK | 0.00 | 8.30E-01 | -1.35 | 1.40E-03 | 1.16 | 2.88E-03 | -0.20 | 5.39E-01 |
| AT1G71940 | (na) | DEELGVISDDDDS(ph)PSGKR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G72390 | (na) | ISTELATPDS(ph)PK | 1.70 | 3.80E-02 | | 1.00E+00 | | 1.00E+00 | -1.45 | 5.17E-02 |
| AT1G72390 | (na) | LSAGGPPQS(ph)PLSSK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.16 | 6.81E-01 |
| AT1G72690 | (na) | S(ph)PNVFER | | 1.00E+00 | 0.58 | 4.52E-02 | -0.29 | 1.00E+00 | | 1.00E+00 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT1G72790 | (na) | SVSS(ph)YPDLR | | 1.00E+00 | 0.59 | 2.69E-01 | -0.50 | 3.83E-01 | | 1.00E+00 |
| AT1G76070 | (na) | GYYS(ph)DDDTR | 0.71 | 1.97E-01 | -0.07 | 4.72E-01 | 0.29 | 2.57E-01 | -0.49 | 3.37E-01 |
| AT1G76070 | (na) | SIFS(ph)FSPASGR | | 1.00E+00 | | 4.72E-01 | | 2.57E-01 | | 1.00E+00 |
| AT1G76660 | (na) | LTAPSS(ph)PDVPYAR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G76850 | (na) | (ac)S(ph)SDSNDLDEDELLQMALK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G76850 | (na) | GSEDTVS(ph)DDKQSVSADDLLALTK | -0.86 | 3.07E-01 | | 1.00E+00 | | 1.00E+00 | 1.92 | 4.58E-02 |
| AT1G76850 | (na) | GSEDTVSDDKQS(ph)VSADDLLALTK | -0.17 | 4.53E-01 | 0.17 | 3.56E-01 | 0.80 | 1.00E+00 | 1.14 | 5.01E-01 |
| AT1G76850 | (na) | LITESSGS(ph)PSK | 1.03 | 2.40E-01 | 0.46 | 7.11E-01 | 0.10 | 7.26E-01 | -0.47 | 5.59E-01 |
| AT1G76850 | (na) | LITESSGS(ph)PSKAEK | 0.38 | 5.62E-02 | -0.77 | 5.46E-04 | 0.23 | 2.24E-01 | -0.92 | 1.51E-04 |
| AT1G76850 | (na) | VALTSLQS(ph)LPR | 0.28 | 6.91E-01 | -0.14 | 4.22E-01 | 0.46 | 2.60E-01 | 0.04 | 9.63E-01 |
| AT1G78880 | (na) | ISGPLDYSGS(ph)M(ox)K | | 1.00E+00 | 0.50 | 2.89E-01 | | 1.00E+00 | 0.91 | 4.00E-01 |
| AT1G78880 | (na) | M(ox)SGNLASAGSNS(ph)M(ox)KK | -2.47 | 4.02E-02 | -0.03 | 9.82E-01 | -1.54 | 6.53E-02 | 0.90 | 1.33E-01 |
| AT1G78880 | (na) | QNS(ph)GPIPILPTTGLITSGPITSGPLNSSGAPR | -1.04 | 2.69E-01 | | 1.00E+00 | | 1.00E+00 | 1.24 | 3.86E-01 |
| AT1G78880 | (na) | S(ph)GAQSGPVPNATGR | 0.28 | 6.45E-01 | 0.05 | 9.98E-01 | 0.25 | 5.61E-01 | 0.03 | 9.10E-01 |
| AT1G79090 | (na) | S(ph)SSSGNYDGM(ox)LGFGDLR | -0.99 | 8.12E-02 | | 1.00E+00 | -0.68 | 2.76E-01 | | 1.00E+00 |
| AT1G79090 | (na) | SSFVSYPPPGSIS(ph)PDQR | | 8.12E-02 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT1G79090 | (na) | SSS(ph)SGNYDGM(ox)LGFGDLR | -1.07 | 1.00E+00 | 0.28 | 2.50E-01 | -0.86 | 1.00E+00 | 0.49 | 1.72E-01 |
| AT1G79150 | (na) | VIPPPLLPPDVAEEDIEFS(ph)DEDLK | | 1.00E+00 | | 2.50E-01 | | 1.00E+00 | | 1.72E-01 |
| AT1G80180 | (na) | NTGRVS(ph)PAVDPPS(ph)PR | -0.01 | 9.47E-01 | 0.90 | 3.69E-02 | -0.14 | 6.92E-01 | 0.78 | 8.70E-02 |
| AT1G80810 | (na) | QLANES(ph)EEETPK | 0.44 | 1.31E-01 | -0.11 | 3.07E-01 | 0.30 | 1.04E-01 | -0.25 | 3.75E-01 |
| AT2G04410 | (na) | SDS(ph)PGKDEPGYNK | 0.36 | 2.91E-02 | -0.26 | 1.64E-01 | -0.13 | 4.37E-01 | -0.75 | 4.93E-04 |
| AT2G07360 | (na) | SYES(ph)DDEEPR | 0.23 | 3.60E-01 | -0.55 | 1.98E-02 | -0.18 | 1.95E-01 | -0.96 | 1.07E-02 |
| AT2G07360 | (na) | SYES(ph)DDEEPRK | 0.03 | 9.38E-01 | -0.33 | 2.91E-01 | 0.20 | 5.65E-01 | -0.16 | 7.07E-01 |
| AT2G07360 | (na) | SYES(ph)DDEEPRKS(ph)TGTR | 0.05 | 9.22E-01 | -0.73 | 2.73E-01 | 0.32 | 5.03E-01 | -0.46 | 5.73E-01 |
| AT2G07360 | (na) | TSS(ph)ISAGPGR | 0.92 | 1.67E-01 | -0.17 | 6.48E-01 | 0.06 | 6.68E-01 | -1.03 | 1.61E-01 |
| AT2G07360 | (na) | YQSTYEGYGS(ph)PIREEPPPPYSYSEPQSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G15860 | (na) | EEEKTEEADTEQDS(ph)DDENAK | -0.10 | 1.49E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G15860 | (na) | LENSVQQGSS(ph)PR | 0.86 | 7.69E-02 | 0.37 | 3.31E-01 | -0.24 | 6.41E-01 | -0.73 | 1.60E-01 |
| AT2G16405 | (na) | TPSLLSS(ph)PGNSGR | | 1.00E+00 | -0.26 | 5.05E-01 | | 1.00E+00 | | 1.00E+00 |
| AT2G16485 | (na) | AIAPPELS(ph)PR | -0.44 | 2.43E-01 | 0.37 | 2.61E-01 | -0.39 | 2.17E-01 | 0.42 | 2.86E-01 |
| AT2G16900 | (na) | ILSLPGS(ph)FGK | 0.17 | 7.91E-01 | 1.43 | 2.91E-02 | -0.82 | 1.04E-01 | 0.45 | 3.97E-01 |
| AT2G16900 | (na) | NDTASEISLFNVVS(ph)PPR | 0.28 | 5.29E-01 | -0.32 | 1.92E-01 | 1.58 | 7.23E-02 | 0.99 | 2.55E-01 |
| AT2G16900 | (na) | TVPLS(ph)PNSMADR | 1.14 | 1.00E+00 | | 1.00E+00 | -0.28 | 1.24E-01 | | 1.00E+00 |
| AT2G18690 | (na) | DVEYM(ox)ALS(ph)STTLTE | | 3.99E-02 | | 1.00E+00 | 1.97 | 5.32E-02 | | 1.00E+00 |
| AT2G20740 | (na) | HYDS(ph)DDEYNVSTVALLQDAR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G20760 | (na) | VTEEKRPS(ph)PAK | | 1.00E+00 | | 5.83E-02 | | 7.91E-02 | | 1.00E+00 |
| AT2G20960 | (na) | RPQT(ph)PETRPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G20960 | (na) | SKT(ph)PEPQPTYFEPSSR | | 1.00E+00 | 0.39 | 1.13E-01 | | 1.00E+00 | -0.78 | 8.83E-02 |
| AT2G20960 | (na) | SKT(ph)PEPSPR | | 1.00E+00 | 0.73 | 3.01E-01 | -3.15 | 2.94E-02 | | 1.00E+00 |
| AT2G22400 | (na) | STDSTEKS(ph)PSKESVVTVDAGVPDESAVEK | | 1.00E+00 | | 6.06E-02 | | 3.35E-01 | | 1.00E+00 |
| AT2G23520 | (na) | DVFETDLLEDNAS(ph)DR | | 1.00E+00 | | 1.00E+00 | 0.89 | 1.66E-01 | | 1.00E+00 |
| AT2G23520 | (na) | EGSLPTVIEEEAEDSET(ph) | 0.17 | 1.00E+00 | | 1.00E+00 | 0.08 | 3.16E-01 | | 1.00E+00 |
| AT2G25430 | (na) | SGVSVNS(ph)GGNSSHHSNNDDR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G25670 | (na) | ESQEQQANNNADAVDEAAGS(ph)EPTEEESPIDVK | -0.72 | 6.83E-02 | | 1.00E+00 | | 1.00E+00 | 0.35 | 2.33E-01 |
| AT2G25730 | (na) | ASFTSSLS(ph)NFQR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G25800 | (na) | S(ph)SSM(ox)SSDLPPSPLGQLAVQLSDSDLR | -0.06 | 4.46E-01 | | 1.00E+00 | | 1.00E+00 | -0.19 | 5.65E-01 |
| AT2G26570 | (na) | FSGS(ph)PVSTGTPK | 0.15 | 7.63E-01 | 0.49 | 4.28E-01 | -0.59 | 3.18E-01 | -0.25 | 5.93E-01 |
| AT2G27090 | (na) | EES(ph)RDSDDDEFDEPTSDTLVR | 0.54 | 2.01E-01 | 0.66 | 9.82E-02 | -0.26 | 5.10E-01 | -0.15 | 7.54E-01 |
| AT2G29140 | (na) | NGT(ph)PDPQGIAR | 0.38 | 2.61E-01 | 0.06 | 4.05E-01 | -0.45 | 6.56E-02 | -0.77 | 6.74E-02 |
| AT2G29200 | (na) | NGT(ph)PDPQAIAR | -0.13 | 3.25E-01 | 0.33 | 7.20E-01 | -0.40 | 5.97E-01 | 0.06 | 1.98E-01 |
| AT2G30530 | (na) | AFLDEDDPNQLPQS(ph)PK | 0.70 | 6.75E-03 | 0.72 | 1.96E-03 | -0.40 | 6.56E-02 | -0.38 | 7.65E-02 |
| AT2G30930 | (na) | ATS(ph)ALSEAK | 0.97 | 9.64E-02 | 1.32 | 3.46E-02 | -1.07 | 8.51E-02 | -0.73 | 2.12E-01 |
| AT2G30930 | (na) | LVGGVTNLVSGASSS(ph)TVANR | 0.13 | 5.43E-01 | 1.02 | 2.90E-01 | 0.66 | 5.15E-01 | 1.55 | 2.15E-01 |
| AT2G30930 | (na) | S(ph)LLQTFEAK | | 1.00E+00 | 1.13 | 3.86E-02 | 0.06 | 3.58E-01 | | 1.00E+00 |
| AT2G32240 | (na) | DIDLSFS(ph)SPTKR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G32240 | (na) | DIDLSFSS(ph)PTK | 0.90 | 6.04E-02 | 0.72 | 2.18E-01 | -0.73 | 1.96E-01 | -0.91 | 5.39E-02 |
| AT2G32910 | (na) | EILVRNS(ph)PDPPDSAVTLDSYR | | 3.18E-01 | | 9.93E-04 | | 2.61E-01 | | 3.16E-03 |
| AT2G32910 | (na) | NS(ph)PDPPDSAVTLDSYR | 1.26 | 1.90E-02 | | 1.00E+00 | -0.44 | 9.93E-01 | | 1.00E+00 |
| AT2G34310 | (na) | DPS(ph)PPPLSSLGK | 3.51 | 1.10E-02 | | 1.00E+00 | | 1.00E+00 | -3.90 | 2.34E-02 |
| AT2G34310 | (na) | DSDGNLSSPGSQGNEEFGTRDPS(ph)PPPLSSLGK | -0.36 | 7.60E-01 | 0.54 | 2.59E-01 | -0.01 | 9.85E-01 | 0.89 | 9.43E-02 |
| AT2G34310 | (na) | S(ph)AEETNKEIK | -0.75 | 8.81E-02 | 1.37 | 1.59E-02 | -0.90 | 5.11E-02 | 1.21 | 2.63E-02 |
| AT2G34310 | (na) | SDEKEEILS(ph)PR | 0.10 | 8.98E-01 | 0.52 | 2.00E-01 | -0.71 | 7.20E-02 | -0.28 | 4.71E-01 |
| AT2G34357 | (na) | KADS(ph)DEEAEFDVEGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G34585 | (na) | SLNPDYDPDDQDVDS(ph)SSDVR | 0.97 | 1.09E-02 | 1.13 | 7.48E-03 | -0.43 | 1.95E-01 | -0.26 | 2.54E-01 |
| AT2G35880 | (na) | (ac)AS(ph)EDLNIVAESK | | 4.80E-01 | | 1.00E+00 | | 1.00E+00 | | 6.86E-03 |
| AT2G35880 | (na) | S(ph)SVGSASGFSFR | | 1.00E+00 | 0.14 | 4.78E-01 | -0.05 | 9.65E-01 | | 1.00E+00 |
| AT2G36630 | (na) | ANM(ox)VNS(ph)RGELLIDTEYEPLYPR | -2.01 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 1.57 | 1.00E+00 |
| AT2G37970 | (na) | ESEKIEMTS(ph)PVVTK | | 1.00E+00 | 0.48 | 1.00E+00 | -0.07 | 4.00E-01 | | 1.00E+00 |
| AT2G37970 | (na) | IEM(ox)TS(ph)PVVTK | 0.04 | 9.37E-01 | 1.24 | 6.74E-02 | -1.19 | 9.03E-02 | 0.01 | 9.26E-01 |
| AT2G38770 | (na) | NM(ox)QQLNQS(ph)PDIDGELSK | 0.55 | 8.00E-02 | 1.06 | 8.28E-03 | -0.96 | 1.67E-02 | -0.45 | 9.74E-02 |
| AT2G40430 | (na) | TELGPAPVPLTIEGDTLS(ph)EDER | 0.06 | 9.15E-01 | -0.46 | 2.11E-01 | 1.14 | 3.78E-02 | 0.62 | 2.66E-01 |
| AT2G40430 | (na) | YFLDVDNFS(ph)EGEDNENVENEVSEAGIK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.93 | 1.00E+00 |
| AT2G40980 | (na) | FQQS(ph)TM(ox)SPEIVEVQEESTK | 0.20 | 7.18E-01 | 0.98 | 7.09E-02 | -0.73 | 2.02E-01 | 0.05 | 9.14E-01 |
| AT2G40980 | (na) | MPS(ph)PVYASSSNALEAK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G43160 | (na) | ADDNS(ph)QDGRGGLQR | 0.87 | 1.41E-01 | -0.21 | 5.40E-01 | 0.21 | 4.78E-01 | -0.87 | 1.61E-01 |
| AT2G43160 | (na) | FSEQNIGAPPSYEEAVSDS(ph)RSPVYSER | -1.30 | 6.18E-02 | | 1.00E+00 | | 1.00E+00 | 1.42 | 7.77E-02 |
| AT2G43160 | (na) | KFS(ph)EQNIGAPPSYEEAVSDSR | -0.59 | 3.69E-01 | -0.84 | 6.61E-02 | 0.49 | 2.04E-01 | 0.24 | 8.41E-01 |
| AT2G43160 | (na) | S(ph)RSVDNYGSR | 1.14 | 2.78E-02 | -0.24 | 4.95E-01 | -0.19 | 7.48E-01 | -1.57 | 7.56E-03 |
| AT2G44440 | (na) | LGDIS(ph)DGENEGAFR | | 1.00E+00 | 0.20 | 4.98E-02 | -0.52 | 1.96E-02 | | 1.00E+00 |
| AT2G44440 | (na) | LGDIS(ph)DGENEGAFRR | | 1.00E+00 | 0.06 | 4.43E-02 | 0.01 | 9.33E-01 | | 1.00E+00 |
| AT2G45540 | (na) | AFKDDDFEQVS(ph)LGDQEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G45540 | (na) | LSS(ph)PGPER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G47980 | (na) | (ac)M(ox)EDS(ph)PQGLKR | 0.04 | 3.94E-01 | -0.59 | 1.99E-01 | 0.16 | 1.55E-01 | -0.48 | 2.94E-01 |
| AT2G47980 | (na) | S(ph)SDQIELDDDDFQETRPKPK | -0.80 | 5.70E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT2G47980 | (na) | SRDPDQDQDDDS(ph)GEAGKADGSGGENQER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.91 | 1.00E+00 |
| AT3G01160 | (na) | NDAS(ph)DDEDEEEEEDEDVINQK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G01780 | (na) | IEEES(ph)ENEEEEEGEEEDDDEEVK | 0.87 | 2.05E-01 | | 1.00E+00 | | 1.00E+00 | 0.02 | 4.23E-01 |
| AT3G01780 | (na) | IEEES(ph)ENEEEEEGEEEDDDEEVKEK | 0.15 | 6.92E-01 | 0.04 | 9.21E-01 | 0.31 | 5.13E-01 | 0.21 | 7.15E-01 |
| AT3G01780 | (na) | IEEES(ph)ENEEEEEGEEEDDDEEVKEKK | 1.42 | 1.12E-01 | | 1.00E+00 | | 1.00E+00 | -0.20 | 3.42E-01 |
| AT3G02860 | (na) | KTEEESPS(ph)DEEDDEDSAVDWR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G03320 | (na) | (ac)M(ox)EQPM(ox)S(ph)PGTK | | 1.00E+00 | | 1.00E+00 | | 6.70E-02 | | 1.00E+00 |
| AT3G03570 | (na) | YSLVLDPNLDAGT(ph)PR | 0.20 | 5.40E-01 | 0.11 | 3.56E-01 | 0.35 | 1.25E-01 | 0.26 | 6.56E-01 |
| AT3G05090 | (na) | GGS(ph)FLAGNLS(ph)FNR | 0.29 | 3.98E-01 | -0.13 | 6.58E-01 | 0.05 | 7.92E-01 | -0.38 | 3.12E-01 |
| AT3G05090 | (na) | TVGS(ph)SNNISVQSSPSHGYTPTIAK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.01 | 4.01E-01 |
| AT3G05090 | (na) | VGS(ph)AGSNGGSVR | -1.22 | 5.05E-02 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G05090 | (na) | WPAEVQS(ph)PK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G05900 | (na) | TSESGSALS(ph)PEK | 1.43 | 2.40E-02 | 1.31 | 4.11E-02 | -0.66 | 1.99E-01 | -0.77 | 1.84E-01 |
| AT3G05900 | (na) | VVPTNQDS(ph)DTEPKKETEGDVPSPADVIEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G06170 | (na) | AGSSTTFLS(ph)PPS(ph)SPR | 0.83 | 1.81E-01 | 1.20 | 7.31E-02 | -0.60 | 1.86E-01 | -0.23 | 5.23E-01 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT3G06550 | (na) | ALLIEDGGGLQSAS(ph)PR | 0.89 | 2.37E-04 | 1.13 | 2.64E-06 | -0.76 | 1.39E-04 | -0.52 | 1.04E-02 |
| AT3G06550 | (na) | DFVKEDDKALLIEDGGGLQSAS(ph)PR | -2.04 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.46 | 1.53E-01 |
| AT3G06550 | (na) | EDDKALLIEDGGGLQSAS(ph)PR | 0.24 | 5.48E-01 | -0.24 | 6.38E-01 | 0.40 | 4.61E-01 | -0.08 | 7.42E-01 |
| AT3G06670 | (na) | ALEKEEEDYFNEDS(ph)DEEDSASASNTQK | -0.24 | 6.26E-01 | -0.32 | 4.34E-01 | 0.62 | 2.28E-01 | 0.55 | 3.24E-01 |
| AT3G06868 | (na) | S(ph)FSGDFFER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G07030 | (na) | VAKPKPES(ph)PINENEIR | | 8.69E-02 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G07660 | (na) | AVDSLPLPRPS(ph)SSEVR | -0.59 | 3.93E-01 | -0.15 | 9.12E-01 | 0.34 | 8.00E-01 | 0.78 | 4.63E-01 |
| AT3G09560 | (na) | FYDFQDDPPS(ph)PTSEYGSAR | 0.32 | 1.01E-01 | -0.02 | 9.12E-01 | 0.64 | 1.65E-02 | 0.30 | 2.85E-01 |
| AT3G10730 | (na) | (ac)SASTVSITAS(ph)PR | | 1.00E+00 | 0.93 | 1.41E-01 | -0.80 | 2.09E-01 | | 1.00E+00 |
| AT3G11330 | (na) | LPS(ph)FTAK | 0.42 | 6.91E-02 | 0.66 | 1.81E-02 | -0.44 | 8.09E-02 | -0.20 | 3.20E-01 |
| AT3G13062 | (na) | VALAYFLS(ph)K | | 4.55E-02 | | 2.55E-03 | | 7.14E-02 | | 2.27E-02 |
| AT3G13360 | (na) | RAS(ph)EERGDIEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.67 | 6.45E-01 |
| AT3G13990 | (na) | NGPPNAHRPS(ph)SPTSK | 0.70 | 1.00E+00 | 0.08 | 3.96E-01 | -0.47 | 1.00E+00 | -1.09 | 1.73E-01 |
| AT3G13990 | (na) | S(ph)DSPVSAVSEPQLPEQK | | 1.00E+00 | 0.72 | 3.96E-01 | -0.62 | 1.00E+00 | | 1.73E-01 |
| AT3G14120 | (na) | M(ox)SQLELQEELSS(ph)P | 0.11 | 5.18E-01 | 0.36 | 3.98E-01 | -0.28 | 5.05E-01 | -0.04 | 6.43E-01 |
| AT3G15160 | (na) | (ac)S(ph)DQEGDWDFYLR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G16270 | (na) | SLT(ph)M(ox)ENENFSR | | 1.00E+00 | -0.34 | 6.97E-01 | -0.82 | 1.51E-01 | | 1.00E+00 |
| AT3G16310 | (na) | SDFSPESGIADYSAS(ph)PDAK | | 1.00E+00 | | 1.00E+00 | -0.24 | 8.44E-01 | | 1.00E+00 |
| AT3G16890 | (na) | ESEAREMFS(ph)S(ph)IER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G17160 | (na) | EKPLVQVLS(ph)DDDS(ph)EVK | | 1.00E+00 | -0.62 | 3.61E-01 | 0.78 | 1.81E-02 | | 1.00E+00 |
| AT3G17160 | (na) | EKPLVQVLS(ph)DDDS(ph)EVKEAK | -1.45 | 1.37E-01 | 0.59 | 6.64E-01 | -0.55 | 5.62E-01 | 1.48 | 2.23E-01 |
| AT3G18230 | (na) | SVAVQQPIDGS(ph)PR | 0.01 | 8.53E-01 | 0.30 | 4.31E-01 | -0.67 | 8.59E-02 | -0.38 | 2.10E-01 |
| AT3G18370 | (na) | ILRGS(ph)PSK | -2.34 | 9.26E-02 | 0.39 | 1.00E+00 | -1.23 | 1.66E-01 | 1.50 | 7.44E-03 |
| AT3G19670 | (na) | LTLTSDLKQPASVPGSS(ph)SPVENVDR | | 1.77E-01 | | 1.00E+00 | | 1.00E+00 | | 2.50E-01 |
| AT3G19895 | (na) | GGGLQAQPVESNS(ph)L | | 1.00E+00 | | 1.00E+00 | -0.37 | 2.42E-01 | | 1.00E+00 |
| AT3G20250 | (na) | ET(ph)DSLSSDAIASEDPFTTDLASQSFTNAQTER | | 1.00E+00 | | 1.00E+00 | | 2.42E-01 | | 1.00E+00 |
| AT3G20250 | (na) | FEQLFGEES(ph)EVS(ph)EEGTEG | 0.46 | 4.76E-01 | | 1.00E+00 | | 1.00E+00 | 0.78 | 4.85E-01 |
| AT3G20250 | (na) | QASHEDNNLSVFGAS(ph)PPSSVASR | 0.29 | 5.79E-01 | | 1.00E+00 | | 1.00E+00 | 0.31 | 4.85E-01 |
| AT3G20250 | (na) | SES(ph)APPSMEGSFAALR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 4.85E-01 |
| AT3G20550 | (mi) | GGS(ph)EEPNVEEDSVAR | 0.82 | 3.27E-02 | -0.06 | 9.69E-01 | 0.14 | 8.29E-01 | -0.74 | 4.45E-02 |
| AT3G21290 | (na) | NVSGLSIGS(ph)SPLDSQR | | 1.00E+00 | | 1.54E-01 | | 1.41E-01 | | 1.00E+00 |
| AT3G21520 | (na) | (ac)S(ph)ETSLLIPK | -0.09 | 9.10E-01 | 0.91 | 6.28E-02 | -0.74 | 1.42E-01 | 0.25 | 6.49E-01 |
| AT3G24080 | (na) | AEGNES(ph)GEDDDFLR | 0.22 | 4.88E-01 | 0.06 | 7.71E-01 | 0.10 | 7.87E-01 | -0.06 | 8.39E-01 |
| AT3G25500 | (na) | STFISIS(ph)PSMS(ph)PK | 0.68 | 3.79E-02 | | 1.00E+00 | 0.13 | 6.27E-01 | | 1.00E+00 |
| AT3G27210 | (na) | GSS(ph)SPAPLPR | 0.63 | 3.00E-01 | 1.21 | 1.09E-01 | -0.26 | 2.73E-01 | 0.32 | 4.64E-01 |
| AT3G27390 | (na) | IFS(ph)QRS(ph)FR | | 1.00E+00 | 0.10 | 9.11E-01 | 0.03 | 8.92E-01 | | 1.00E+00 |
| AT3G27390 | (na) | LSEDLDLKDNNS(ph)AKDESITEPPAPVK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G27390 | (na) | SSNAISAS(ph)PPLTER | 0.14 | 2.65E-01 | 0.72 | 1.66E-02 | -0.29 | 2.28E-01 | 0.29 | 1.24E-01 |
| AT3G29075 | (na) | DGNNS(ph)EDDEFKK | | 1.00E+00 | -0.16 | 6.82E-01 | 0.21 | 7.26E-01 | | 1.00E+00 |
| AT3G29075 | (na) | DGNNS(ph)EDDEFKKK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G29075 | (na) | KDGNNS(ph)EDDEFKK | 0.85 | 5.57E-02 | -1.10 | 4.06E-02 | 0.36 | 3.91E-01 | -1.58 | 8.88E-03 |
| AT3G44190 | (na) | (ac)M(ox)EKTES(ph)VSGK | -0.15 | 9.20E-01 | -0.17 | 6.15E-01 | 0.43 | 3.25E-01 | 0.40 | 5.81E-01 |
| AT3G47200 | (na) | (ac)ADKTDIISSSS(ph)DKASPPPPSAFR | | 1.00E+00 | | 1.00E+00 | 0.31 | 1.00E+00 | | 1.00E+00 |
| AT3G47210 | (na) | (ac)AEKTEIFSSPS(ph)SPPPSFYMVDK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.38 | 1.00E+00 |
| AT3G47210 | (na) | LSS(ph)PSLPSS(ph)PPPSYYM(ox)GEK | 0.08 | 8.78E-01 | 1.15 | 8.31E-02 | -1.19 | 4.99E-02 | -0.13 | 8.41E-01 |
| AT3G47210 | (na) | LSSPSLPS(ph)SPPPSYYM(ox)GEK | 0.05 | 7.34E-01 | 1.24 | 3.67E-02 | -0.71 | 1.85E-01 | 0.48 | 4.42E-01 |
| AT3G47210 | (na) | TEIFSSPS(ph)SPPPSFYM(ox)VDK | 0.94 | 7.49E-02 | 0.42 | 3.68E-01 | 0.30 | 4.24E-01 | -0.22 | 7.20E-01 |
| AT3G47210 | (na) | YSSPSS(ph)PPPSFYR | 1.03 | 4.30E-02 | 1.42 | 2.95E-02 | -0.49 | 4.78E-01 | -0.10 | 6.29E-01 |
| AT3G49210 | (na) | KDDEEEVEEEQPLS(ph)PAAR | 1.01 | 1.15E-01 | | 1.00E+00 | | 1.00E+00 | -0.85 | 1.54E-01 |
| AT3G49590 | (na) | FSGVLSS(ph)SDSPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G49590 | (na) | IITDYVGS(ph)PATDPM(ox)R | -0.19 | 3.53E-01 | 0.99 | 2.08E-01 | -1.45 | 8.08E-02 | -0.27 | 1.92E-01 |
| AT3G49590 | (na) | S(ph)PSSQDSLPGIALYR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.32 | 2.49E-01 |
| AT3G49590 | (na) | YISGGNS(ph)PR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 2.49E-01 |
| AT3G49601 | (na) | AARDS(ph)DDS(ph)EIEYQNKK | 0.83 | 1.26E-01 | -0.51 | 4.37E-01 | 0.51 | 1.00E+00 | -0.83 | 6.48E-01 |
| AT3G49800 | (na) | S(ph)SDLAEGDEDLIAGIR | -0.24 | 9.64E-01 | -0.38 | 1.00E+00 | 0.89 | 6.53E-02 | 0.75 | 3.48E-01 |
| AT3G50370 | (na) | NPLS(ph)PNSGQANLK | | 1.00E+00 | -0.15 | 1.00E+00 | 1.12 | 1.00E+00 | | 1.00E+00 |
| AT3G51890 | (na) | (ac)SSTLS(ph)NEESGLGDSNR | 0.37 | 4.76E-01 | 0.74 | 1.46E-01 | -0.56 | 3.32E-01 | -0.18 | 7.06E-01 |
| AT3G52230 | (na) | EATGDDDQKDDDEDDQSS(ph)DGHED | 1.10 | 1.42E-01 | 0.12 | 8.66E-01 | 0.44 | 4.38E-01 | -0.54 | 1.18E-01 |
| AT3G52230 | (na) | KEATGDDDQKDDDEDDQS(ph)SDGHED | 1.31 | 2.89E-02 | | 1.00E+00 | | 1.00E+00 | -1.28 | 2.35E-01 |
| AT3G54760 | (na) | AASQM(ox)AGS(ph)PSVLK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.05 | 9.83E-01 |
| AT3G54760 | (na) | KDNADM(ox)TSDLTGTIESADSAIPNS(ph)PTEDAAPGNIQDLK | -1.22 | 6.42E-02 | | 1.00E+00 | | 1.00E+00 | 0.78 | 1.96E-01 |
| AT3G55600 | (na) | IEDEPRS(ph)PT(ph)SPQLR | | 1.00E+00 | 1.03 | 7.58E-02 | -1.36 | 1.00E+00 | | 1.00E+00 |
| AT3G55600 | (na) | SPTS(ph)PQLR | 0.87 | 4.11E-02 | 1.29 | 1.16E-01 | -0.22 | 4.23E-01 | 0.20 | 6.38E-01 |
| AT3G55970 | (sec) | VQS(ph)LSESNLGAIPNR | | 1.00E+00 | 1.14 | 1.01E-02 | -1.13 | 4.18E-02 | | 1.00E+00 |
| AT3G56720 | (na) | (ac)M(ox)DTELNS(ph)PPHDDGGDTTTAFR | 0.25 | 2.26E-01 | 0.42 | 7.71E-02 | -0.32 | 1.60E-01 | -0.16 | 4.66E-01 |
| AT3G58110 | (na) | (ac)AS(ph)SPPSDPTDRDAETLSQNPSLIEKPSVVEQGSLSVENVAEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G58110 | (na) | (ac)ASSPPSDPT(ph)DRDAETLSQNPSLIEKPSVVEQGSLSVENVAEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G60450 | (na) | (ac)MES(ph)PKSNVDDGYQNIFVM(ox)R | 0.16 | 4.69E-01 | 0.23 | 1.08E-01 | 0.41 | 6.23E-03 | 0.48 | 2.05E-01 |
| AT3G61480 | (na) | DIEQAPTES(ph)DSLSPK | | 1.81E-01 | | 1.00E+00 | | 1.00E+00 | | 4.13E-01 |
| AT3G61480 | (na) | QIGLTST(ph)SSPK | 0.04 | 9.97E-01 | -0.03 | 9.41E-01 | 0.03 | 9.59E-01 | -0.03 | 9.76E-01 |
| AT3G61690 | (na) | S(ph)SPELTETHGEALLQSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G62900 | (na) | GIS(ph)SPLVAGNHVISAQNR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G63160 | (na) | DKS(ph)DSDDATVPPPSGA | 0.39 | 7.35E-01 | 1.62 | 7.10E-02 | -0.75 | 4.96E-01 | 0.49 | 3.38E-01 |
| AT3G63460 | (na) | DFM(ox)PS(ph)DTDFSTK | 0.11 | 9.39E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT3G63460 | (na) | DFMPS(ph)DTDFSTK | 0.65 | 3.36E-01 | | 1.00E+00 | | 1.00E+00 | -0.27 | 1.00E+00 |
| AT3G63460 | (na) | DQAVDGLS(ph)SDLNGIR | 0.63 | 8.57E-02 | 0.72 | 9.15E-02 | -0.06 | 9.25E-01 | 0.04 | 9.58E-01 |
| AT4G01150 | (na) | AS(ph)SEETSSIDTNELITDLK | -0.12 | 9.08E-01 | -0.53 | 1.52E-01 | 0.31 | 5.80E-02 | -0.10 | 7.63E-01 |
| AT4G01290 | (na) | S(ph)IGELSQR | 0.31 | 3.78E-01 | | 1.00E+00 | | 1.00E+00 | 0.82 | 5.46E-02 |
| AT4G05150 | (na) | ASS(ph)ISSLLDSSVNR | -0.19 | 8.50E-01 | 0.30 | 1.69E-01 | 0.47 | 5.72E-01 | 0.96 | 2.03E-01 |
| AT4G05150 | (na) | EVSTLS(ph)DPGS(ph)PR | 0.13 | 4.52E-01 | 0.32 | 4.49E-01 | -0.14 | 6.05E-01 | 0.05 | 3.11E-01 |
| AT4G05150 | (na) | EVSTLSDPGS(ph)PRR | 0.30 | 3.43E-01 | 0.54 | 6.79E-02 | -1.01 | 2.24E-02 | -0.76 | 2.08E-01 |
| AT4G05150 | (na) | IST(ph)PELPPPVFIKPES(ph)PEPVSTPK | 0.26 | 5.60E-01 | -2.01 | 1.77E-02 | 1.60 | 3.13E-02 | -0.67 | 2.83E-01 |
| AT4G08330 | (na) | APEYALVTQNSDPT(ph)SPR | 0.25 | 6.78E-01 | | 1.00E+00 | -0.40 | 7.29E-01 | | 1.00E+00 |
| AT4G08330 | (na) | APEYALVTQNSDPTS(ph)PR | -0.29 | 1.65E-01 | 0.90 | 2.02E-03 | -0.81 | 1.48E-02 | 0.37 | 1.58E-01 |
| AT4G08540 | (na) | LDSPGS(ph)NSFK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G10750 | (na) | DVDDEKYWS(ph)E | | 1.00E+00 | 0.45 | 2.33E-01 | 0.05 | 1.00E+00 | | 1.00E+00 |
| AT4G11790 | (na) | QDHSDDADGGDEQSQPSS(ph)PSVK | 0.79 | 1.20E-01 | | 1.00E+00 | | 1.00E+00 | 0.27 | 1.00E+00 |
| AT4G11860 | (na) | S(ph)FDDSPPAAAELR | 1.45 | 7.78E-02 | 1.09 | 2.25E-01 | -0.24 | 3.79E-01 | -0.60 | 2.54E-01 |
| AT4G11860 | (na) | S(ph)FDDSPPAAAELRR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G11860 | (na) | T(ph)RSFDDSPPAAAELR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G12070 | (na) | FQEIPFTNES(ph)L | | 1.00E+00 | -0.36 | 1.00E+00 | 0.61 | 2.12E-01 | | 1.00E+00 |
| AT4G14280 | (na) | (ac)VDRPTDVVVEVGS(ph)SGR | 0.77 | 1.00E+00 | 0.94 | 3.10E-03 | 0.72 | 4.35E-02 | 0.88 | 2.26E-03 |
| AT4G14465 | (na) | QIHGGGDS(ph)PPR | 1.81 | 1.00E+00 | 0.41 | 1.28E-01 | 0.51 | 2.47E-01 | -0.89 | 1.00E+00 |
| AT4G15545 | (na) | (ac)S(ph)EIEEEEEEGSASAITGSR | 0.37 | 2.19E-01 | 1.02 | 2.17E-01 | -0.09 | 3.91E-01 | 0.56 | 1.79E-01 |
| AT4G15545 | (na) | TTSRPIS(ph)PR | | 1.00E+00 | 0.28 | 4.62E-02 | -0.25 | 2.94E-01 | | 1.00E+00 |
| AT4G15610 | (na) | GYETKST(ph)LDTER | -0.33 | 1.23E-01 | | 1.00E+00 | | 1.00E+00 | 0.16 | 3.48E-01 |
| AT4G15790 | (na) | (ac)ADQTSDSTSPVAPLS(ph)VSSPTATKK | -0.35 | 1.00E+00 | 2.38 | 1.06E-03 | -0.01 | 1.72E-01 | 2.71 | 1.00E+00 |
| AT4G15790 | (na) | (ac)ADQTSDSTSPVAPLSVS(ph)SPTATKK | | 1.00E+00 | -0.66 | 1.37E-01 | | 1.00E+00 | -0.45 | 7.83E-02 |
| AT4G15802 | (na) | AEM(ox)GVEGT(ph)PPPASK | -0.24 | 3.48E-01 | 0.09 | 4.51E-01 | -1.17 | 2.19E-01 | -0.85 | 1.69E-01 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT4G17060 | (na) | VSADFDADS(ph)DDEIVLVPK | -0.92 | 9.47E-03 | -1.28 | 1.60E-03 | 1.01 | 1.16E-02 | 0.64 | 4.63E-02 |
| AT4G17140 | (na) | ASGSAPIAGLSDTSS(ph)DSEESETEQK | 1.23 | 8.84E-02 | 0.92 | 5.31E-02 | 0.06 | 8.91E-01 | -0.24 | 2.35E-01 |
| AT4G17140 | (na) | ELSSSSS(ph)VSDDKK | 0.37 | 2.44E-01 | 0.17 | 6.58E-01 | -0.10 | 7.57E-01 | -0.31 | 3.48E-01 |
| AT4G17140 | (na) | LDPTSS(ph)EGEEK | 1.19 | 9.94E-02 | 0.93 | 5.66E-02 | -0.12 | 6.97E-01 | -0.38 | 1.47E-01 |
| AT4G17140 | (na) | SFIQSSEM(ox)LPSFEDAES(ph)RSPER | -1.37 | 5.19E-02 | | 1.00E+00 | | 1.00E+00 | 0.52 | 5.45E-01 |
| AT4G17140 | (na) | SFIQSSEM(ox)LPSFEDAES(ph)RSPERLDPTSSEGEEK | -2.19 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.98 | 1.04E-02 |
| AT4G17140 | (na) | TPS(ph)FSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.04E-02 |
| AT4G17330 | (na) | KPEDQNISGFM(ox)SAGS(ph)PVLNR | -0.13 | 4.34E-01 | -0.29 | 7.67E-01 | 1.02 | 1.27E-02 | 0.87 | 5.87E-01 |
| AT4G17330 | (na) | TDDGQEQVLVKDDS(ph)PTAVEEASVEEK | -1.41 | 4.34E-01 | | 1.00E+00 | | 1.27E-02 | 0.88 | 1.00E+00 |
| AT4G17620 | (na) | DLFGS(ph)DNEEYTK | 1.34 | 1.00E+00 | 0.61 | 3.82E-01 | -0.33 | 4.74E-01 | -1.06 | 1.00E+00 |
| AT4G18070 | (na) | SQGDKES(ph)DDGVNER | 0.38 | 2.66E-01 | -0.10 | 4.97E-01 | -0.08 | 9.25E-01 | -0.56 | 1.05E-01 |
| AT4G18120 | (na) | SPIFGNLS(ph)PTK | | 1.00E+00 | 0.45 | 6.74E-02 | 0.20 | 2.41E-01 | | 1.00E+00 |
| AT4G18905 | (na) | NVEGS(ph)NEDEEEEIEEDGEEISEVDHAK | | 1.00E+00 | | 6.74E-02 | | 2.41E-01 | 1.51 | 1.00E+00 |
| AT4G19490 | (na) | SIS(ph)DASSQSLSSILNNPHGGK | | 1.00E+00 | | 1.00E+00 | | 6.56E-02 | | 1.00E+00 |
| AT4G22270 | (na) | GNAIET(ph)SDDEEGEGDDDLDNTK | | 1.00E+00 | | 1.00E+00 | -0.60 | 1.64E-01 | | 1.00E+00 |
| AT4G22670 | (na) | SFVVEES(ph)DDDMDETEEVKPK | -0.21 | 9.83E-01 | -0.39 | 4.53E-01 | 0.61 | 1.93E-01 | 0.44 | 5.98E-01 |
| AT4G22670 | (na) | VEEEEEEDEIVES(ph)DVELEGDTVEPDNDPPQK | -0.45 | 4.78E-01 | -1.09 | 2.92E-03 | 1.62 | 3.17E-04 | 0.98 | 9.68E-02 |
| AT4G22980 | (na) | FTSQES(ph)LPR | 0.25 | 3.32E-01 | | 1.00E+00 | | 1.00E+00 | 0.49 | 6.40E-01 |
| AT4G24275 | (na) | S(ph)VSASAQAVPSPIK | -0.01 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.25 | 2.81E-01 |
| AT4G24680 | (na) | SSLNAWGTSSLS(ph)PR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G24840 | (na) | (ac)SDLVATS(ph)PSPSSAPR | 0.61 | 1.25E-01 | 0.57 | 2.71E-01 | 0.00 | 9.91E-01 | -0.04 | 6.85E-01 |
| AT4G24840 | (na) | (ac)SDLVATSPS(ph)PSSAPR | 0.43 | 1.61E-01 | 0.49 | 1.06E-01 | -0.25 | 4.20E-01 | -0.19 | 5.21E-01 |
| AT4G25070 | (na) | FDDES(ph)EDDETR | -0.23 | 2.75E-01 | | 1.00E+00 | | 1.00E+00 | -0.46 | 3.08E-01 |
| AT4G25070 | (na) | FDDEYDS(ph)DEITEDVPR | 0.14 | 6.02E-01 | | 1.00E+00 | | 1.00E+00 | 0.33 | 6.66E-01 |
| AT4G25070 | (na) | FDDEYDS(ph)DEITEDVPRNPVEIR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G25770 | (na) | LYEQPGEVDSLDS(ph)PSKEK | -0.27 | 4.19E-01 | 0.46 | 2.74E-01 | -0.73 | 6.68E-02 | -0.01 | 8.95E-01 |
| AT4G25880 | (na) | IPS(ph)PPIYYPTEYQFIDNR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G25880 | (na) | LS(ph)PGSQSLADFR | | 1.00E+00 | 0.49 | 3.85E-01 | 0.84 | 2.04E-01 | | 1.00E+00 |
| AT4G25880 | (na) | S(ph)GSAPPNM(ox)EGSFLAVDNLLSR | | 1.00E+00 | -1.33 | 3.43E-02 | -0.56 | 1.00E+00 | | 1.00E+00 |
| AT4G26130 | (na) | TTS(ph)IGDGGEEGVDDK | 0.84 | 1.88E-01 | 0.00 | 9.17E-01 | 0.29 | 6.06E-01 | -0.56 | 3.63E-01 |
| AT4G26130 | (na) | TTS(ph)IGDGGEEGVDDKASNFINK | -0.30 | 8.59E-01 | -0.21 | 5.58E-01 | -0.13 | 9.22E-01 | -0.03 | 7.92E-01 |
| AT4G26630 | (na) | AEVDESKVEDEKEGS(ph)EDENDNEK | -0.55 | 2.44E-01 | 0.67 | 4.05E-01 | -0.18 | 9.89E-01 | 1.04 | 1.00E+00 |
| AT4G26630 | (na) | SLAHS(ph)DDES(ph)EEEKEEEEK | | 1.00E+00 | -0.65 | 3.72E-01 | | 1.00E+00 | 0.14 | 3.67E-01 |
| AT4G26630 | (na) | SLAHS(ph)DDES(ph)EEEKEEEEKQEEEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.18 | 2.78E-01 |
| AT4G26630 | (na) | VEDEKEGS(ph)EDENDNEK | 0.65 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.09 | 2.78E-01 |
| AT4G26630 | (na) | VEDEKEGS(ph)EDENDNEKVESK | -0.08 | 7.17E-01 | 0.04 | 9.18E-01 | 0.11 | 7.20E-01 | 0.23 | 9.14E-01 |
| AT4G27500 | (na) | KTGGNTETETEEVPEAS(ph)EEEIEAPVQEEKPQK | | 2.17E-01 | | 1.19E-01 | | 5.48E-02 | | 1.73E-01 |
| AT4G27500 | (na) | TGGNTETETEEVPEAS(ph)EEEIEAPVQEEKPQK | -0.72 | 6.90E-02 | -0.07 | 8.15E-01 | 0.71 | 2.64E-02 | 1.36 | 5.81E-04 |
| AT4G27585 | (na) | DHQETQALDET(ph)DLEELEDM(ox)GEK | -0.34 | 4.02E-01 | | 1.00E+00 | 0.29 | 1.00E+00 | | 1.00E+00 |
| AT4G27870 | (na) | GS(ph)AAELTEPTTGTEK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G27900 | (na) | AFS(ph)ESDIQTLGTGNTGLVQSQLDR | -0.34 | 1.70E-01 | | 1.00E+00 | | 1.00E+00 | 0.18 | 6.06E-01 |
| AT4G28080 | (na) | DAGDSNSGLS(ph)PKPK | 1.17 | 5.75E-03 | 0.20 | 5.09E-01 | 0.17 | 6.53E-01 | -0.80 | 3.16E-02 |
| AT4G28080 | (na) | ESGSTDGDSPTEKDAGDSNSGLS(ph)PKPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G28080 | (na) | STNFTS(ph)PR | -0.51 | 8.57E-02 | -0.85 | 9.30E-03 | 0.14 | 5.74E-01 | -0.20 | 3.19E-01 |
| AT4G28080 | (na) | TVDGETENLPNGDS(ph)SPK | -0.05 | 8.92E-01 | 0.43 | 1.47E-01 | -0.59 | 1.09E-01 | -0.10 | 7.00E-01 |
| AT4G29440 | (na) | FDDYDRDS(ph)ES(ph)EEDNLGR | -0.22 | 5.12E-01 | 0.55 | 2.04E-01 | 0.01 | 7.58E-01 | 0.78 | 1.37E-01 |
| AT4G29440 | (na) | FGPLASGLENETTLPSYGS(ph)SPPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G29440 | (na) | FGPLASGLENETTLPSYGSS(ph)PPRDK | -0.86 | 2.40E-01 | | 1.00E+00 | | 1.00E+00 | 0.79 | 4.56E-01 |
| AT4G31130 | (na) | EDHFDEVES(ph)R | -0.31 | 4.91E-02 | 0.16 | 3.31E-01 | 0.18 | 8.52E-02 | 0.65 | 1.47E-01 |
| AT4G31160 | (na) | QLTFSPS(ph)FSSQSR | 0.27 | 3.27E-01 | 0.55 | 4.19E-02 | -0.54 | 5.70E-02 | -0.26 | 2.70E-01 |
| AT4G31160 | (na) | VHEGAPDTEVLLAS(ph)PR | -0.37 | 8.08E-01 | 0.59 | 4.99E-01 | 0.14 | 9.85E-01 | 1.09 | 3.49E-01 |
| AT4G31880 | (na) | (ac)SDS(ph)DKELENQIIEAGEK | 1.96 | 1.47E-01 | 0.32 | 5.96E-01 | -0.17 | 5.45E-01 | -1.80 | 5.44E-04 |
| AT4G31880 | (na) | AAEIST(ph)PER | 0.86 | 4.02E-02 | | 1.00E+00 | | 1.00E+00 | -0.65 | 2.82E-01 |
| AT4G31880 | (na) | QTVEES(ph)PNSNTK | 0.46 | 7.43E-01 | 0.48 | 1.36E-01 | -0.30 | 3.20E-01 | -0.28 | 7.59E-01 |
| AT4G31880 | (na) | QTVEES(ph)PNSNTKR | 1.07 | 4.57E-02 | -2.10 | 5.17E-03 | 1.54 | 6.44E-03 | -1.63 | 1.12E-02 |
| AT4G31880 | (na) | TSGDETANVSS(ph)PSM(ox)AEELPEQSVPK | | 1.00E+00 | -0.07 | 4.02E-01 | | 1.00E+00 | -0.52 | 4.12E-02 |
| AT4G31880 | (na) | TSGDETANVSS(ph)PSM(ox)AEELPEQSVPKK | -0.71 | 1.70E-01 | 0.03 | 3.65E-01 | -0.12 | 3.77E-01 | 0.62 | 3.17E-02 |
| AT4G31880 | (na) | WSPLDESELS(ph)QDEEAADQTGQEEDASTVPLTK | -0.45 | 1.95E-01 | -3.69 | 6.19E-06 | 3.27 | 3.07E-03 | 0.04 | 9.11E-01 |
| AT4G32180 | (Co) | SGS(ph)RPQLDLSK | -1.05 | 8.03E-03 | | 1.00E+00 | | 1.00E+00 | 0.77 | 3.90E-02 |
| AT4G32285 | (na) | S(ph)FGDVNEIGAR | 1.28 | 8.29E-02 | 0.68 | 5.65E-01 | -0.20 | 8.77E-01 | -0.80 | 1.62E-01 |
| AT4G32285 | (na) | S(ph)RSFGDVNEIGAR | -2.55 | 1.28E-01 | 1.25 | 2.05E-01 | -3.56 | 6.35E-02 | 0.24 | 3.11E-01 |
| AT4G32285 | (na) | SRS(ph)FGDVNEIGAR | 0.66 | 1.90E-01 | -1.86 | 5.55E-03 | 2.05 | 1.71E-03 | -0.47 | 4.44E-01 |
| AT4G35110 | (na) | LLS(ph)FS(ph)GSFGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G35140 | (na) | SSSS(ph)PEREGESSSATGR | 1.46 | 5.27E-02 | -1.51 | 1.47E-01 | 1.33 | 1.46E-01 | -1.64 | 1.02E-01 |
| AT4G35240 | (na) | FVASGGANVGDS(ph)PR | 0.35 | 3.69E-01 | 0.44 | 5.75E-01 | 0.15 | 8.68E-01 | 0.24 | 2.23E-01 |
| AT4G35890 | (na) | (ac)ASATSNNPASSS(ph)MSPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G35890 | (na) | GESEPIAAAAAVAGPS(ph)SPQSR | 0.43 | 2.30E-01 | 0.98 | 7.05E-02 | -0.54 | 1.17E-01 | 0.01 | 2.42E-01 |
| AT4G36630 | (na) | GSSGISDDM(ox)ESSS(ph)PR | 0.27 | 1.55E-01 | 0.13 | 9.85E-01 | -0.45 | 3.61E-01 | -0.58 | 2.51E-01 |
| AT4G36850 | (na) | SLAGS(ph)GT(ph)PPLR | 0.48 | 3.03E-01 | 0.96 | 7.49E-02 | -0.72 | 9.98E-02 | -0.24 | 5.38E-01 |
| AT4G36850 | (na) | TM(ox)GSAIS(ph)IPGGSYK | | 9.06E-02 | | 1.00E+00 | | 1.00E+00 | | 4.74E-01 |
| AT4G37120 | (na) | NNNENGDDATS(ph)DGEEDLDDLRVDEAK | 0.11 | 5.77E-01 | 0.65 | 2.21E-02 | 0.03 | 2.95E-01 | 0.57 | 2.23E-01 |
| AT4G37700 | (na) | IDEAS(ph)PLISFGSK | 0.82 | 1.86E-01 | 0.35 | 3.50E-01 | 0.75 | 1.22E-01 | 0.28 | 3.71E-02 |
| AT4G38360 | (na) | GIDDPLLNGSFS(ph)DSGVTR | 0.17 | 3.68E-01 | -0.09 | 3.91E-01 | -0.07 | 3.23E-01 | -0.33 | 1.03E-01 |
| AT4G38550 | (na) | EQFEDLYEQDGDVT(ph)PR | 0.20 | 1.00E+00 | -0.53 | 3.29E-01 | 0.12 | 3.14E-01 | -0.61 | 3.88E-02 |
| AT4G38550 | (na) | EQIEDFYEQDDDVT(ph)PR | 0.28 | 6.49E-02 | 0.16 | 3.09E-01 | 0.17 | 2.94E-01 | 0.05 | 8.69E-01 |
| AT4G38550 | (na) | S(ph)PPPSYLSNK | 0.21 | 1.88E-01 | -0.22 | 1.14E-01 | 0.11 | 3.29E-01 | -0.33 | 7.93E-02 |
| AT4G38550 | (na) | S(ph)PPPSYLSNKR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT4G38550 | (na) | SNYDKEQFEDLYEQDGDVT(ph)PR | -0.46 | 2.09E-01 | | 1.00E+00 | | 1.00E+00 | 0.04 | 8.70E-01 |
| AT5G02850 | (na) | LGLTGPGS(ph)PSVQNPTPTR | 0.58 | 1.25E-03 | 0.74 | 9.62E-02 | -0.15 | 7.77E-01 | 0.01 | 3.33E-01 |
| AT5G03700 | (na) | NLGSDS(ph)FNSVEM(ox)SR | 0.66 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G03700 | (na) | VLEEDNGLS(ph)PGPYK | 0.50 | 4.61E-02 | 0.85 | 1.32E-01 | -0.56 | 3.26E-01 | -0.21 | 1.44E-01 |
| AT5G04420 | (na) | LKTES(ph)SSADNIQEDDGSSLR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G04420 | (na) | QRS(ph)ASDEEEDGTVQR | 1.16 | 2.23E-02 | | 1.00E+00 | | 1.00E+00 | -2.14 | 1.27E-02 |
| AT5G04420 | (na) | SAS(ph)DEEEDGTVQR | 0.60 | 3.05E-01 | 0.09 | 9.89E-01 | -0.07 | 8.51E-01 | -0.58 | 3.53E-01 |
| AT5G04420 | (na) | TES(ph)SSADNIQEDDGSSLR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G04550 | (na) | M(ox)SDFLSGSLS(ph)AESPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G04990 | (na) | (ac)S(ph)ASTVSITANTAAATR | -0.32 | 1.16E-01 | 0.62 | 1.73E-01 | -0.47 | 2.83E-01 | 0.48 | 4.73E-02 |
| AT5G04990 | (na) | GEATLDRS(ph)QGQDLGPVTR | 0.19 | 5.26E-01 | 0.41 | 8.97E-02 | -0.21 | 1.33E-01 | 0.01 | 8.86E-01 |
| AT5G04990 | (na) | S(ph)QGQDLGPVTR | | 1.00E+00 | | 8.97E-02 | | 1.33E-01 | | 1.00E+00 |
| AT5G04990 | (na) | SVS(ph)AATGTNTTATQR | | 1.00E+00 | -1.50 | 1.00E+00 | | 1.00E+00 | -0.27 | 2.38E-01 |
| AT5G05190 | (na) | AQANEPQSVPETNNVSSSSGQDTVLPSS(ph)PGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.08 | 4.40E-01 |
| AT5G05600 | (sec) | VQS(ph)LAESNLSSLPDR | -0.18 | 4.72E-01 | 1.32 | 8.42E-05 | -0.79 | 4.20E-03 | 0.71 | 1.50E-02 |
| AT5G06440 | (na) | NITPVPETEELS(ph)PNRADDQAK | -0.09 | 6.09E-01 | 0.71 | 3.19E-01 | -0.45 | 4.89E-01 | 0.35 | 8.51E-01 |
| AT5G06560 | (na) | LDFSNDS(ph)PR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -1.29 | 1.00E+00 |
| AT5G06560 | (na) | NPS(ph)FTQPTGDVSGGR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G06970 | (na) | SES(ph)FDSPK | 0.26 | 5.68E-01 | 0.03 | 8.53E-01 | -0.20 | 8.88E-01 | -0.43 | 3.98E-01 |
| AT5G08050 | (na) | SGFSLST(ph)IER | 0.46 | 2.95E-01 | -0.02 | 9.83E-01 | -0.89 | 6.04E-02 | -1.38 | 1.05E-01 |
| AT5G08660 | (na) | S(ph)QDFDSEYSR | 0.55 | 1.89E-01 | 0.53 | 9.37E-02 | 0.05 | 4.36E-01 | 0.02 | 8.70E-01 |
| AT5G08660 | (na) | S(ph)SEYFR | 0.06 | 4.79E-01 | 0.59 | 1.66E-01 | -0.22 | 4.59E-01 | 0.31 | 9.92E-02 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT5G09390 | (na) | SAANDTEMDDDS(ph)GNEKAGDDLSQDEIGVR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 1.23 | 4.99E-02 |
| AT5G09620 | (na) | FSYNSYPDS(ph)AESSPR | 0.33 | 2.00E-01 | 0.98 | 1.00E+00 | -1.52 | 1.37E-02 | -0.88 | 1.00E+00 |
| AT5G09960 | (na) | AS(ph)LGNEEELIKPPESATSTAELTTVQSENQR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G09960 | (na) | DPS(ph)RASLGNEEELIKPPESATSTAELTTVQSENQR | -0.95 | 2.01E-01 | | 1.00E+00 | | 1.00E+00 | 1.14 | 3.67E-01 |
| AT5G10950 | (na) | ADKDEDEEENET(ph)S(ph)DDEAEPK | 0.94 | 6.58E-03 | -0.21 | 4.13E-01 | 0.37 | 1.42E-01 | -0.78 | 1.69E-02 |
| AT5G11040 | (na) | LPVLDGSFFTKDPPPGS(ph)PSSSR | -0.21 | 9.62E-01 | -1.18 | 1.00E+00 | 1.56 | 7.50E-03 | 0.58 | 3.96E-01 |
| AT5G11970 | (na) | SYS(ph)ASYGTR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G13020 | (na) | EIS(ph)PGDIR | 0.49 | 9.94E-01 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G13020 | (na) | LEEIS(ph)DGESGNI | 0.38 | 1.90E-01 | 0.62 | 6.41E-02 | -0.45 | 1.62E-01 | -0.21 | 4.06E-01 |
| AT5G13260 | (na) | LSDIELKS(ph)PGGPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G13560 | (na) | QQFESIARPTLEIEIPS(ph)PR | -3.34 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 1.79 | 1.84E-01 |
| AT5G14240 | (na) | VVKDHEDKDNDDGGYNS(ph)D | 2.22 | 1.00E+00 | -0.14 | 3.03E-01 | 0.73 | 1.00E+00 | -1.63 | 6.69E-03 |
| AT5G15680 | (na) | S(ph)ASGPLSPM(ox)PPELNIVPVATGR | 0.50 | 3.10E-01 | -1.13 | 1.37E-01 | 0.14 | 4.86E-01 | -1.49 | 1.04E-01 |
| AT5G15680 | (na) | S(ph)GQLLPSLVNASGPLM(ox)GVR | | 3.10E-01 | | 1.37E-01 | | 4.86E-01 | | 1.04E-01 |
| AT5G15680 | (na) | TLVPQTS(ph)FK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G15680 | (na) | VPFGYS(ph)PADPR | | 1.00E+00 | 1.05 | 2.65E-02 | 0.23 | 3.27E-01 | | 1.00E+00 |
| AT5G15680 | (na) | VPFGYS(ph)PADPRT(ph)PSYSK | | 1.00E+00 | 0.74 | 2.79E-01 | -0.76 | 5.31E-01 | | 1.00E+00 |
| AT5G19390 | (na) | GFVADDS(ph)DIES(ph)PRDTNGPR | -0.60 | 1.46E-01 | -0.45 | 1.00E+00 | 0.09 | 1.00E+00 | 0.24 | 2.94E-01 |
| AT5G21010 | (na) | (ac)SESVIQGSNPDRVLS(ph)PTSSK | -0.96 | 1.00E+00 | 0.39 | 1.00E+00 | -0.49 | 1.00E+00 | 0.87 | 1.00E+00 |
| AT5G21160 | (na) | STSAETIGDGDKDS(ph)PK | 0.73 | 3.29E-01 | | 1.00E+00 | | 1.00E+00 | -0.81 | 2.97E-01 |
| AT5G23060 | (na) | S(ph)GTKFLPSSD | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.27 | 4.82E-01 |
| AT5G23060 | (na) | SGT(ph)KFLPSSD | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G23890 | (na) | SPVPESTDGSKDELNIYS(ph)QDELDDNR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 1.09 | 7.33E-02 |
| AT5G24290 | (na) | SLS(ph)DSEEKSNLEK | | 1.00E+00 | -0.63 | 3.59E-01 | -0.23 | 6.21E-01 | | 1.00E+00 |
| AT5G24890 | (na) | S(ph)FGNLGEIGSVK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G24890 | (na) | SKS(ph)FGNLGEIGSVK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.78 | 1.00E+00 |
| AT5G26610 | (na) | VAIASVFGNDS(ph)DED | | 1.00E+00 | 0.30 | 2.70E-01 | -0.60 | 7.47E-02 | | 1.00E+00 |
| AT5G35180 | (na) | QSS(ph)TLVNDVR | -0.15 | 6.39E-01 | 0.59 | 1.01E-01 | -1.01 | 2.62E-02 | -0.28 | 4.25E-01 |
| AT5G35430 | (na) | TSS(ph)LLSSSVASDTLR | 0.04 | 8.21E-01 | 1.39 | 6.14E-03 | -1.38 | 3.75E-03 | -0.03 | 9.12E-01 |
| AT5G39570 | (na) | KKYGGNDS(ph)DEDEEK | 8.08 | 2.89E-05 | 1.15 | 1.41E-01 | -0.41 | 8.01E-01 | -7.34 | 1.02E-04 |
| AT5G39570 | (na) | KYGGNDS(ph)DEDEEK | -1.54 | 1.20E-02 | -1.33 | 1.00E+00 | 0.26 | 1.00E+00 | 0.47 | 3.89E-02 |
| AT5G39570 | (na) | NRS(ph)GSGDDEEGSYGRK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G39570 | (na) | NRSGS(ph)GDDEEGSYGR | 0.09 | 7.46E-01 | -0.30 | 7.82E-01 | -1.32 | 2.26E-01 | -1.71 | 5.41E-02 |
| AT5G39570 | (na) | SEEQEEGS(ph)YR | -0.13 | 9.16E-01 | 0.28 | 8.74E-01 | -0.39 | 8.65E-01 | 0.02 | 9.25E-01 |
| AT5G39570 | (na) | SGS(ph)GDDEEGSYGR | 0.31 | 6.51E-01 | -0.04 | 8.00E-01 | 0.14 | 7.25E-01 | -0.21 | 7.15E-01 |
| AT5G39570 | (na) | SGS(ph)GDDEEGSYGRK | 0.57 | 1.85E-01 | -0.38 | 1.02E-01 | 0.21 | 2.40E-01 | -0.74 | 8.03E-02 |
| AT5G39570 | (na) | YGGNDS(ph)DEDEEK | -0.39 | 8.32E-01 | 1.11 | 3.96E-01 | -0.46 | 9.20E-01 | 1.04 | 3.81E-01 |
| AT5G39570 | (na) | YGGNDS(ph)DEDEEKK | -0.76 | 1.30E-01 | 1.16 | 3.57E-01 | -0.69 | 4.89E-01 | 1.23 | 1.55E-02 |
| AT5G39570 | (na) | YGGNDS(ph)DEDEEKKK | -3.46 | 4.05E-11 | -0.07 | 4.07E-01 | 0.04 | 4.74E-01 | 3.43 | 5.11E-11 |
| AT5G40300 | (na) | QDQS(ph)SPINFEM(ox)SSR | | 1.00E+00 | 0.01 | 3.90E-01 | -0.85 | 3.94E-01 | | 1.00E+00 |
| AT5G40450 | (na) | S(ph)LSDHIQK | | 1.00E+00 | -0.34 | 1.00E+00 | 0.26 | 4.66E-01 | | 1.00E+00 |
| AT5G40640 | (na) | IFS(ph)QKS(ph)FK | | 1.00E+00 | | 1.00E+00 | | 4.66E-01 | | 1.00E+00 |
| AT5G40640 | (na) | NEEGASTAFSGGLS(ph)RPNSFK | | 1.00E+00 | | 1.00E+00 | | 4.66E-01 | | 1.00E+00 |
| AT5G40640 | (na) | NEEGASTAFSGGLSRPNS(ph)FK | | 1.00E+00 | | 1.00E+00 | | 4.66E-01 | | 1.00E+00 |
| AT5G41950 | (na) | NLS(ph)GKAETM(ox)STNVER | 0.04 | 5.26E-01 | 0.43 | 6.78E-01 | -1.18 | 2.29E-01 | -0.78 | 1.64E-01 |
| AT5G42860 | (na) | TDSEVTSLSAS(ph)S(ph)PTRSPR | 1.11 | 4.57E-02 | 0.33 | 3.51E-01 | -0.07 | 8.60E-01 | -0.85 | 3.00E-02 |
| AT5G42870 | (na) | LDLREDESS(ph)SGGLDAESVAESSPK | | 4.57E-02 | | 1.00E+00 | | 1.00E+00 | | 3.00E-02 |
| AT5G42870 | (na) | LVGS(ph)LPIM(ox)R | | 1.00E+00 | 0.91 | 2.38E-02 | -0.90 | 2.48E-02 | | 1.00E+00 |
| AT5G42920 | (na) | KYEDDLDLVLDDDS(ph)EIDEPTGR | -1.45 | 4.14E-02 | | 1.00E+00 | | 1.00E+00 | 0.43 | 1.35E-01 |
| AT5G45510 | (na) | EDT(ph)DGEDEIR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G45510 | (na) | GNPSDQESSS(ph)ESPKK | -2.16 | 2.50E-02 | -0.14 | 4.68E-01 | 0.30 | 4.71E-02 | 2.32 | 1.58E-02 |
| AT5G45510 | (na) | KEDT(ph)DGEDEIR | 1.11 | 3.08E-02 | 0.40 | 2.62E-01 | 0.35 | 5.39E-01 | -0.36 | 4.16E-01 |
| AT5G45510 | (na) | SGEHAEGEANDS(ph)QSGEK | | 1.00E+00 | 2.57 | 1.75E-02 | -0.38 | 4.68E-01 | | 1.00E+00 |
| AT5G47480 | (na) | ESNIVDGSGS(ph)PGVK | 0.56 | 4.17E-01 | 0.85 | 2.11E-01 | -0.26 | 5.42E-01 | 0.03 | 9.79E-01 |
| AT5G47480 | (na) | SEVDDM(ox)ALTETGKESNIVDGS(ph)GSPGVK | | 1.00E+00 | 0.03 | 1.00E+00 | | 1.00E+00 | | 6.30E-02 |
| AT5G47480 | (na) | SVS(ph)EPDFSR | -0.11 | 3.38E-01 | -0.82 | 3.77E-02 | 0.20 | 2.90E-01 | -0.51 | 8.70E-02 |
| AT5G47690 | (na) | EAEENAETS(ph)DNETLGAWK | 0.30 | 5.56E-01 | -0.36 | 7.95E-01 | 0.41 | 3.46E-01 | -0.24 | 8.81E-01 |
| AT5G47690 | (na) | EAEHDDS(ph)DTEGKQENNEM(ox)ER | 0.96 | 1.02E-03 | -0.43 | 1.20E-01 | 0.27 | 2.20E-01 | -1.12 | 6.10E-04 |
| AT5G47690 | (na) | LESHADASVIPQTSENEVM(ox)IDGES(ph)DGNEIPLGK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | 0.96 | 7.08E-02 |
| AT5G47690 | (na) | M(ox)LESIS(ph)PR | | 1.00E+00 | 0.32 | 4.27E-01 | -0.62 | 2.06E-01 | | 1.00E+00 |
| AT5G47690 | (na) | SSALPIETEYSGEAGEEKS(ph)ESEGK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G48240 | (na) | DYNS(ph)DDDEEEDDESKKQPEVTIR | | 1.00E+00 | 0.53 | 7.92E-02 | -0.53 | 1.11E-01 | | 1.00E+00 |
| AT5G48370 | (na) | (ac)M(ox)NS(ph)PRPISVVSTFASPSSTSDPTR | -0.19 | 8.87E-01 | 0.07 | 8.74E-01 | 0.34 | 5.18E-01 | 0.61 | 3.24E-01 |
| AT5G50640 | (na) | S(ph)SISLSGER | -0.29 | 1.89E-01 | 0.15 | 7.23E-01 | -0.57 | 1.59E-01 | -0.13 | 2.69E-01 |
| AT5G52200 | (na) | TVSGTS(ph)S(ph)SSSPELI | | 3.08E-03 | | 1.00E+00 | | 1.00E+00 | | 3.33E-02 |
| AT5G53420 | (na) | AFS(ph)EGDIQK | 0.06 | 7.64E-01 | 0.55 | 5.04E-01 | -1.03 | 1.27E-01 | -0.54 | 4.84E-01 |
| AT5G53420 | (na) | LGAGLVQS(ph)PLDR | 1.23 | 5.05E-02 | 0.90 | 2.78E-01 | -0.16 | 8.68E-01 | -0.48 | 3.64E-01 |
| AT5G53440 | (na) | DGRRS(ph)PDYQDYQDVITGSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G53440 | (na) | EVAELS(ph)GGS(ph)ER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G53440 | (na) | KGANDGEEAEADDGDGDGSVVVGDVS(ph)PK | 0.51 | 2.80E-01 | 0.29 | 5.43E-01 | -0.41 | 3.54E-01 | -0.62 | 1.08E-01 |
| AT5G53440 | (na) | S(ph)PDYQDYQDVITGSR | 0.06 | 7.60E-01 | 0.40 | 4.31E-01 | -0.55 | 1.55E-01 | -0.21 | 3.49E-01 |
| AT5G53440 | (na) | S(ph)PGTENYTEKR | -0.16 | 2.97E-01 | -0.37 | 3.77E-02 | -0.14 | 3.03E-01 | -0.35 | 4.46E-02 |
| AT5G53800 | (na) | GS(ph)DRDGAPPENTKR | -2.28 | 2.26E-03 | -0.46 | 2.13E-01 | 0.35 | 3.09E-01 | 2.17 | 3.01E-03 |
| AT5G54440 | (na) | AS(ph)LSTGNIPEM(ox)FDGRPSFTEGSGLEASPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G54440 | (na) | TNS(ph)SPGNFESPLDRPM(ox)R | 0.77 | 9.04E-02 | 1.18 | 1.81E-02 | -0.29 | 4.52E-01 | 0.12 | 8.11E-01 |
| AT5G55210 | (na) | DSEIEETEEFDTES(ph)PLPK | -0.06 | 7.84E-01 | -0.25 | 2.21E-01 | 0.72 | 7.49E-03 | 0.52 | 2.60E-02 |
| AT5G55210 | (na) | VISKDSEIEETEEFDTES(ph)PLPK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G55660 | (na) | SVAHS(ph)DDES(ph)EEEKEDDEEEEK | 0.29 | 1.00E+00 | -1.38 | 1.00E+00 | 0.92 | 1.00E+00 | -0.75 | 1.00E+00 |
| AT5G55660 | (na) | TAVPTKS(ph)SPPKK | | 1.00E+00 | -1.21 | 1.00E+00 | | 1.00E+00 | -0.72 | 2.20E-01 |
| AT5G55660 | (na) | TAVPTKSS(ph)PPK | 1.28 | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -1.96 | 3.88E-02 |
| AT5G55860 | (na) | M(ox)AS(ph)ESSPQQHYK | | 1.00E+00 | 0.13 | 5.09E-01 | 0.51 | 2.51E-02 | | 1.00E+00 |
| AT5G55860 | (na) | RDS(ph)SDSSPIVEVGEIDTSAPFQSVK | | 1.00E+00 | | 1.00E+00 | | 2.51E-02 | | 1.00E+00 |
| AT5G55860 | (na) | VLM(ox)PNLS(ph)GIFNR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G56980 | (na) | APS(ph)ILER | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G56980 | (na) | STS(ph)FGDGEDGVDAK | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | -0.49 | 4.96E-01 |
| AT5G57000 | (na) | SLS(ph)PEKER | 1.11 | 1.66E-02 | 0.26 | 4.69E-01 | 0.10 | 7.79E-01 | -0.76 | 6.53E-02 |
| AT5G57370 | (na) | RRS(ph)PS(ph)PDAPSR | 1.03 | 9.66E-03 | -0.22 | 3.73E-01 | 0.31 | 2.46E-01 | -0.94 | 1.43E-02 |
| AT5G57830 | (na) | DASLETPILS(ph)PK | 1.15 | 1.36E-02 | 0.88 | 3.08E-01 | -0.54 | 5.43E-01 | -0.80 | 1.22E-02 |
| AT5G57830 | (na) | NLS(ph)SPFDGISSER | 0.57 | 4.92E-02 | 0.59 | 3.42E-02 | -0.35 | 1.83E-01 | -0.33 | 2.19E-01 |
| AT5G58220 | (na) | VISETDSS(ph)SSPVSTKPQAEAK | 1.38 | 1.00E+00 | 0.93 | 6.56E-02 | -1.40 | 4.40E-02 | -1.84 | 1.00E+00 |
| AT5G59960 | (na) | VSS(ph)PSSSFSR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G60620 | (na) | LVT(ph)SKSELDLDHPNIEDYLPSGSSINEPR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G61450 | (na) | SFSSVPS(ph)SPR | 0.59 | 1.38E-01 | 0.10 | 8.56E-01 | 0.22 | 4.91E-01 | -0.26 | 9.23E-02 |
| AT5G62820 | (na) | YVS(ph)PEGS(ph)PFKIENPK | 1.37 | 1.36E-02 | 0.46 | 4.23E-01 | -0.43 | 4.12E-01 | -1.34 | 7.56E-02 |
| AT5G63220 | (na) | VPVPPHLVDVS(ph)DDEDVQNLQESLGEAR | -1.36 | 2.57E-01 | | 1.00E+00 | | 1.00E+00 | 0.72 | 7.56E-01 |
| AT5G63550 | (na) | (ac)ATETLDEKTPEVNS(ph)PAKEEIDVVPK | -0.58 | 5.52E-01 | -0.02 | 4.50E-01 | 0.13 | 2.54E-01 | 0.69 | 3.97E-01 |
| AT5G63550 | (na) | DKEDDVES(ph)EEEEEEEEGSGSK | 1.35 | 1.96E-01 | 1.20 | 2.50E-01 | -0.25 | 1.27E-01 | -0.40 | 3.79E-01 |
| AT5G63550 | (na) | DKEDDVES(ph)EEEEEEEEGSGSKK | | 1.96E-01 | | 1.00E+00 | | 1.27E-01 | | 1.00E+00 |
| AT5G63550 | (na) | TPEVNS(ph)PAKEEIDVVPK | -0.33 | 7.83E-01 | 0.52 | 4.05E-02 | -0.60 | 3.06E-01 | 0.25 | 4.83E-01 |

| | | | ahk1.m/wt.m | | ahk1/wt | | ahk1.m /ahk1 | | wt.m /wt | |
|---|---|---|---|---|---|---|---|---|---|---|
| accession | category | pPeptide | log2 | p-value | log2 | p-value | log2 | p-value | log2 | p-value |
| AT5G63550 | (na) | TPEVNS(ph)PAKEEIDVVPKEEK | | 1.00E+00 | | 4.05E-02 | | 1.00E+00 | | 4.83E-01 |
| AT5G63550 | (na) | VLEFLES(ph)PKETR | | 1.00E+00 | | 4.05E-02 | | 1.00E+00 | | 4.83E-01 |
| AT5G64010 | (na) | GALLQDS(ph)EEEDG | | 1.00E+00 | -0.21 | 5.37E-01 | 0.19 | 5.04E-01 | | 1.00E+00 |
| AT5G64090 | (na) | S(ph)LEIEEDFDR | | 1.00E+00 | | 1.00E+00 | 1.25 | 3.14E-02 | | 1.00E+00 |
| AT5G64160 | (na) | (ac)SLTLLQGYS(ph)SAEEEEAEER | | 1.00E+00 | -0.77 | 1.00E+00 | | 3.14E-02 | | 1.00E+00 |
| AT5G64160 | (na) | AFGDYDNS(ph)DEDGDNDVR | | 1.00E+00 | 0.05 | 3.24E-01 | -0.82 | 3.83E-02 | | 1.00E+00 |
| AT5G64160 | (na) | AFGDYDNS(ph)DEDGDNDVRR | 0.60 | 2.40E-01 | -0.24 | 2.85E-01 | 0.16 | 3.27E-01 | -0.67 | 1.73E-01 |
| AT5G64430 | (na) | FSYNSYPDSTDS(ph)SPR | 0.68 | 1.49E-02 | 0.62 | 1.55E-02 | -0.43 | 8.76E-02 | -0.50 | 5.49E-02 |
| AT5G64430 | (na) | LFLFPASS(ph)GFGSQSSTQSDRDR | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 | | 1.00E+00 |
| AT5G64500 | (na) | YNEDS(ph)EPDAVTR | 1.06 | 6.15E-02 | 1.18 | 4.62E-02 | -0.32 | 5.12E-01 | -0.19 | 8.50E-01 |
| AT5G65687 | (na) | DS(ph)PAKEEAPPATK | 0.29 | 3.57E-01 | 0.17 | 6.86E-01 | -0.08 | 9.65E-01 | -0.20 | 5.04E-01 |
| AT5G65687 | (na) | SNEVS(ph)EDDEVEEDKLESK | 1.03 | 9.39E-02 | 0.34 | 5.55E-01 | 0.18 | 7.47E-01 | -0.52 | 3.90E-01 |
| AT5G65687 | (na) | VGQRDS(ph)PAKEEAPPATK | 0.69 | 2.29E-01 | -0.24 | 8.93E-01 | 0.50 | 7.93E-01 | -0.43 | 2.80E-01 |
| AT5G65950 | (na) | INLVDVGGGGLFS(ph)PR | -0.45 | 2.85E-01 | -1.24 | 1.38E-01 | 1.45 | 1.00E+00 | 0.67 | 4.35E-01 |
| AT5G65950 | (na) | T(ph)SSFRDPLSVSDSASPIPSR | -1.54 | 1.03E-02 | | 1.00E+00 | | 1.00E+00 | 1.96 | 2.56E-02 |
| AT5G66950 | (na) | QGTLPTVIEEDDSSET(ph) | 0.06 | 1.50E-01 | 1.11 | 1.75E-02 | -0.73 | 6.11E-02 | 0.33 | 2.36E-01 |
| AT5G67470 | (na) | LVTGGGDGGGS(ph)RR | | 1.00E+00 | -0.18 | 2.44E-01 | -0.27 | 5.78E-01 | | 1.00E+00 |

# 9 ACKNOWLEDGEMENTS

First of all I thank my supervisor Klaus Harter for the opportunity to work at the ZMBP in his group in this topic for my Ph.D. thesis, for his trust in my work and his continuous support of my ideas as well as his critical remarks. I thank him for giving me the opportunity to work in other labs and for his recommendation and trust to visit meetings and conferences to exchange ideas with other scientists. Furthermore I'd like to express my gratitude to him for the great working atmosphere and the motivating discussions.

In addition, I thank Claudia Oecking and Sabine Müller for their support and especially for their evaluation of my experiments and my ideas as scientists who were not directly involved in my project.

My sincere gratitude goes to Waltraud X. Schulze for the generation of the phosphoproteome data-sets, her help with the analysis, her patience for my questions and inspiring discussions.

For the possibility to work for three weeks in his lab at the University of Nottingham, England, I thank Malcolm J. Bennett as well as Daniela Dietrich and Kamal Swarup of his lab who kindly teached me the hydrotropism-, gravitropism- and thigmotropism-assay as well as the method to analyze lateral root development and who also helped me afterwards to solve various problems.

My gratitude also goes to Gerhard Obermeyer who gave me the opportunity to work for three weeks in his lab at the University of Salzburg, Austria and who teached me to perform the protoplast swelling assay.

Furthermore, I thank Michael Hothorn for the generation of the homology model of the extracellular domain of AHK1, Christa Testerink andher Ph.D. student Dorota Kawa for the execution of the halotropism and RSA assay and Christopher Grefen for the explanation and support in the flame photometric analysis of the ion content in dried plant material.

In addition I thank Thilo Stehle and especially Volker Niemann for the patient and excited trial of CD-spectroscopy with and Erik Nöldeke for the trial of chrystallization of the extracellular domain of AHK1.

My special gratitude goes to Johanna Schröter, Gert Huber, Sofia Riegger and Richard Gavidia who took diligently care of my plants in the greenhouse.

I particularly thank Anne Mohrholz, Michael Heunemann, Stefan Fischer, Juan Suarez, Natalie Krieger, Nina Glöckner, Andreas Hecker, Sachie Kimura, Luise Brand, Üner Kolukisaoglu and Klaus Harter for a great time in the lab, for serious, funny, ridiculous, inspiring, critical, motivating, subject-specific and subject-distant conversations, discussions and outings as well as for the support in experimental execution and the placement of overnight cultures or plant plates to the respective incubator during the weekend.

My cordial gratitude goes to my father Kurt, my sisters Nathalie and Philippa and to their boy-friends Johannes and Sven who supported, motivated, tolerated and encouraged me during my whole work.